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Endolaryngeal CO_2 Laser Microsurgery of Early Vocal Cord Cancer

A Retrospective Study

M. Csanády, J. Czigner, L. Sávay

ENT Department Albert Szent-Györgyi Medical School of Szeged
(Director: Prof. *J. Czigner*), Hungary

Endolaryngeal laser excision of early-stage vocal cord cancer is a discussed, but accepted procedure [1, 2]. This modality for the removal of vocal cord tumor has proved more effective than conservative surgery or radiotherapy [1, 3–6]. The indication must be established by an experienced oncologist [7–9]. The laser excision allows minimal invasive therapy at an early tumor stage (T1, T2), but has also been reported in laser therapy for T3 glottic cancer [9–12]. In the present study, 51 patients with early vocal cord cancer underwent CO_2 laser microsurgery and were followed up for 2–6.5 years.

Materials and Methods

A Storz laryngoscope was used for exploration and a Tungsram CO_2 laser set was applied at a power setting of 5–15 W in continuous mode and coupled to an Opton microscope combined with a micromanipulator. Laser excision was performed under general anesthesia. The vocal cord cancers were excised en bloc with an appropriate tumor-free margin. Fifty-one patients unterwent CO_2 laser cordectomy in the period May 1987 to December 1991. During the laser intervention frozen section histological control was performed to detect incomplete resection. The complications were very moderate. In 3 cases, bleeding from the paraglottic region occurred. One patient had subcutaneous emphysema and another had perichondritis. In 1 case, the protective cotton caught fire, but this was extinguished immediately. Some moderate postoperative edema was observed, but no tracheotomy was needed after the procedure. For the follow-up, we used an indirect laryngoscope, a laryngofiberoscope and biopsy in cases of a suspicion of tumor recurrence. The duration of hospitalization was on average 5 days. Complete healing took 5–6 weeks.

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Table 1. Results after CO_2 laser cordectomy

	Tumor stage				
	T in situ	T1a	T1b	T2	total
Number of patients	7	32	9	3	51
Free of tumor after CO_2 laser excision					
n	7	28	6	2	43
%	*100*	*87*	*67*	*67*	*84*
Incomplete resection	–	1	–	–	1
Recurrence of tumor	–	3	3	1	7
Free of tumor after follow-up	7	30	9	3	49
Irradiation	–	3	1	–	4
Partial laryngectomy	–	1	2	1	4
Laryngectomy	–	1	–	–	1
Died of tumor	–	2	–	–	2
Died of unrelated disease	1	1	1	–	3

Results

Of the 51 vocal cord cancers excised by CO_2 laser, 7 were T in situ vocal cord cancer; after the CO_2 laser procedure, there was no evidence of recurrence. Twenty-eight of the 32 patients with T1a tumor had no recurrence after laser surgery. Four patients required additional therapy for recurrent tumor; laser resection was incomplete and 10 days later partial laryngectomy was performed. One patient required radiotherapy; the tumor recurred and total laryngectomy was then performed. All these patients are free of tumor. Two patients were irradiated after local tumor recurrence, but died later in consequence of the repeatedly recurring tumor or the developing metastases. There were 9 patients in the T1b subgroup. Six of them have been free from tumor recurrence since then. Three patients had recurrent tumor; 1 of them was irradiated (TeCo full course) after local recurrence, and 2 underwent partial laryngectomy. All of the patients in this subgroup are free of tumor. There were also 3 cases of stage T2 vocal cord tumor. In one patient, recurrence developed and partial laryngectomy was performed. All 3 patients have since remained free of tumor (table 1).

Discussion

The role of CO_2 laser excision of vocal cord cancer has increased recently, and our experience with laser microsurgery is likewise good. Surgery of vocal cord lesions requires skilled surgeons, selected patients and a well-defined indication. Endoscopic laser microsurgery is a preferred surgical intervention for the patient, because of the time factor and the many advantages of laser excision. However, there are difficulties during laser surgery particularly as regards excision in the anterior commissure and definition of the exact margins of the excised tumor. Clarification from this aspect requires the help of a pathologist to perform an intraoperative frozen section histological control. In the early stage of vocal cord cancers, our statistics revealed a success rate of 100% for T in situ, 87% for T1a, and 67% for T1b and T2 tumors, which demonstrates the efficacy of this surgical intervention. Including salvage surgery, the healing result was 94%. A comparison of the modalities of treatment of vocal cord cancer shows that the rate of recurrence is not higher than after conventional surgery or radiotherapy, and the voice quality is good in most cases.

References

1 Strong MS, Jakó GJ: Laser surgery in the larynx. Early clinical experience with continuous CO_2 laser. Ann Otol Rhinol Laryngol 1972;81:791–798.
2 Glanz H, Kimmlich T, Eichhorn T, Kleinsasser O: Behandlungsergebnisse bei 584 Kehlkopfkarzinomen an der Hals-Nasen-Ohrenklinik der Universität Marburg. HNO 1989;37:1–10.
3 Jakó GJ: Laser surgery of vocal cords. Laryngoscope 1972;82:2204–2209.
4 Davis RK, Jako GJ, Hyams VJ, Shapshay SM: The anatomical limitations of CO_2 laser chordectomy. Laryngoscope 1982;92:980–984.
5 Mendenhall WM, Parsons JT, Stringer SP, Cassisi NJ, Million RR: T1-T2 vocal cord carcinoma: A basis for comparing the results of radiotherapy and surgery. Head Neck Surg 1988;10:373–377.
6 Steiner W, Iro H, Petsch S, Sauerbrei W: Lasermikrochirurgische Behandlung von Larynxkarzinomen (pT2–4) Darstellung der Langzeitergebnisse; in Dühmke E, Steiner W, Reck R (eds): Funktionserhaltende Therapie des fortgeschrittenen Larynxzkarzinoms. Stuttgart, Thieme, 1991, pp 80–91.
7 Shapshay SM, Hybels RL, Bohigian RK: Laser excision of early vocal cord carcinoma: Indications, limitations and precautions. Ann Otol Rhinol Laryngol 1990;99:46–49.
8 Eckel HE, Thumfart WF: Laser surgery for the treatment of larynx carcinomas: Indications, techniques and preliminary results. Ann Otol Rhinol Laryngol 1992;101:113–118.
9 Thumfart WF: Early cancer of larynx. Lasersurgery of larynx-carcinomas: indications, techniques, follow up; Johnson JT, Didolkar MS (eds): Head and Neck Cancer. Amsterdam, Excerpta Medica Elsevier, 1993, vol 3, pp 215–222.
10 Steiner W, Aurbach G, Ambroch P: Minimally invasive therapy in otolaryngology and head and neck surgery. Minim Invas Ther 1991;1:57–70.
11 Motta G, Villari G, Motta G, Jr, Salerno G: The CO_2 laser in laryngeal microsurgery. Acta Otolaryngol (Stockh) 1986:(suppl 433).
12 Hirano M, Hirade Y: CO_2 laser for treating glottic carcinoma. Acta Otolaryngol 1988; (suppl 458): 154–157.

M. Csanády, MD, ENT Department, Albert Szent-Györgyi Medical School,
Tisza Lajos krt. 111, H–6725 Szeged (Hungary)

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Laryngeal papilloma in adults and CO_2 laser therapy

M. Csanády[1], J. Czigner[1] and L. Tiszlavicz[2]
[1]*Department of Otorhinolaryngology; and* [2]*Institute of Pathology, Albert Szent Györgyi Medical University, Szeged, Hungary*

Abstract. *Background.* Laryngeal papillomatosis is a recurrent disease, its etiology is based on virus detection by polymerase chain reaction (PCR) or immunohistochemical method.

Methods. Twenty-four patients with laryngeal papilloma were treated by the authors and CO_2 laser was used for removal of papilloma. The endolaryngeal approach with the use of CO_2 laser proved to be a very sufficient intervention for fast and accurate excision or vaporization of papilloma. No adjuvant therapy was administered. Avidin-biotin complex (ABC) technique was applied for the detection of human papilloma virus (HPV).

Results. In this retrospective study, patients were divided into juvenile and adult papilloma groups, according to the case history and immunohistochemical findings. Koilocytes were characteristic for juvenile papillomas. Only two patients had been observed in childhood, but four patients with juvenile papillomas were subsequently treated in adulthood.

Conclusions. 61% of the adult group and 100% of childhood papillomas recurred after CO_2 laser surgery. The time intervals between recurrences became longer ranging from 6 months to 5 years. CO_2 laser microsurgery proved to be a safe method for removal of papilloma, nevertheless, it is only a symptomatic treatment.

Keywords: CO_2 laser, laryngomicrosurgery, larynx, papilloma.

Introduction

Laryngeal papillomas are frequently located on the vocal cords, but also anywhere in the larynx. They can also be either solitary or multiple in the treatment of this disease. There are different methods, but the surgical intervention is widely accepted with or without adjuvant therapy. Previously, many kinds of treatment were reportedly applied, including: antibiotics, autogen vaccine [1], hormones and local application of podophyllin [2].

Recently, a laryngomicroscopic approach [3,4] proved to be a good method for the removal of papillomas. Many kinds of surgical interventions were reported including cryotherapy [5] and argon laser [6].

Endolaryngeal use of CO_2 laser with laryngomicroscopy [7,8] is a fast and safe method. Photodynamic therapy (PDT) was also introduced as a successful treatment of papillomas [9]. Efficacy of adjuvant therapy is controversially discussed in literature, but antiviral [10], interferon [11] and ultrasound [12] therapy have been reported as beneficial. Especially, acyclovir, interferon and isoprinosine,

Address for correspondence: Dr Miklós Csanády, Department of Otorhinolaryngology, Albert Szent Györgyi Medical University, Szeged, Tisza L. krt. 111, 6725-Hungary. Tel./Fax: +36-62-310-141.

which can result in longer recurrence intervals, were administered [13—15]. A British study [16] showed no effect of acyclovir on recurrence whereas a Spanish one described it as an effective substance [10]. In vitro experiments with transforming growth factor (TGF-β) inhibited the growing process of papilloma tissue [17]. For extension of papilloma, sonography was described as a good diagnostic method [18].

The etiology of laryngeal papilloma is based on the human papilloma virus (HPV), which has been detected in papilloma tissues. Papillomas are divided into juvenile and adult types. The difference between these groups can be determined by immunohistochemistry, case history, and the type of human papilloma virus.

The human papilloma virus has been detected and classified by DNS hybridization and polymerase chain reaction (PCR). In juvenile papilloma only 6 and 11 types of human papilloma virus (HPV) have been found, in adult papilloma HPV 16 and 18 have also been recognized besides 6 and 11 types [19,20]. HPV 16, 18 could also be found in epidermal carcinoma, and they could have a role in malignant transformation [19,21], though there were only two malignant transformations in a study of 150 cases, in spite of HPV 16, 18 detected in 92 cases.

Laryngeal papilloma recurs in many patients, especially juvenile types. Some cases can be healed at puberty, when hormones are thought to influence the growth of papilloma tissue. Some malignant transformations of adult papillomas have been reported, but the recurrence rate of this type is comparatively low.

We report on a retrospective study about our experiences with the classification and treatment of laryngeal papilloma.

In this retrospective study the immunohistochemical examination contributed to the determination of human papilloma virus (HPV) by detection of capsid protein of HPV [22], because the difference between juvenile and adult forms of papilloma cannot be made by histological examination.

Material and Methods

In a period of 10 years 24 patients with laryngeal papillomas underwent endolaryngeal CO_2 laser surgery at the Clinic of Otolaryngology, Albert Szent Györgyi Medical School, Szeged, Hungary [7]. Under general anaesthesia laryngomicroscopy was performed, and CO_2 laser equipment (Tungsram, TLS 61) coupled to the microscope (Opton) was used for excision (10—15 W) and vaporization (5—10 W). There was no adjuvant therapy, because of reported controversial results. In this study six patients with juvenile and 18 patients with adult papilloma are presented. Only two juvenile onset papillomas were treated since their childhood, the others were first admitted to our clinic after several surgical interventions in other hospitals (Tables 1 and 2).

To distinguish the difference between juvenile and adult papillomatosis the avidin-biotin complex (ABC) immunohistochemical method was used [22], and all the juvenile papillomas and some of the adult papillomas were investigated retrospectively in the Institute of Pathology. The polyclonal antibody of HPV produced

Table 1. Recurrences of laryngeal papillomas.

Papilloma	Patients	Recurrences
Juvenile	6	6 (100%)
Adult	18	11 (61%)
Total	24	17 (70%)

in rabbit was applied and the virus was detected by immunohistochemical reaction. This compound (Biogenex) exhibits a steady reaction, but the serotypes of papilloma virus cannot be differentiated. The intensity of reaction was classified into three groups (O, +, ++), and examinations in the samples taken by the first and last surgery were performed retrospectively. The detection of koilocytes is characteristic for juvenile papilloma.

Results

In the last 10 years 24 patients with laryngeal papilloma were operated on with the endolaryngeal use of CO_2 laser. The analysis of these patients revealed a comparison between the two types of papilloma and presented the value of CO_2 laser in the endoscopic microsurgery (Figs. 1 and 2).

All the juvenile papillomas recurred (100%), three cases of developed repeated recurrences and each of the other six patients suffered from one recurrence only.

Two patients with juvenile papilloma had earlier been tracheotomized because of suffocation, but we managed to remove the cannula after multiple laser surgery. Moreover, possible future recurrences may necessitate ordinary controls and CO_2 laser interventions for maintaining a sufficient airpassage.

The interval between recurrences ranged from 6 months up to 5 years. During the 10 years only two new cases with juvenile onset papilloma were presented.

Juvenile papilloma tended to recur more frequently than the adult type and showed the same histological findings even in elderly patients.

Immunohistochemical examination [22] revealed + and ++ intensity of virus de-

Table 2. Juvenile papillomas treated with CO_2 laser.

Patients	Sex	Age	Age at first surgery	No. of surgical interventions	Localization	Tracheotomy
1	male	27	9	30	multifocal	decannulation[a]
2	male	19	19	2	solitary	—
3	female	30	7	20	multifocal	decannulation[b]
4	female	61	10	8	multifocal	—
5	male	14	9	2	solitary	—
6	female	13	11	2	solitary	—

[a]Due to multiple laser interventions; [b]decannulated after introduction of CO_2 laser surgery.

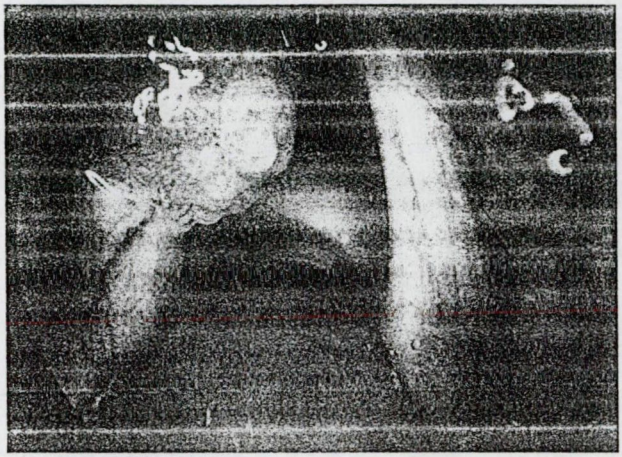

Fig. 1. Adult laryngeal papilloma.

tection in juvenile papillomas and koilocytes were also found. There was no sign of dysplasia.

Adult papillomas recurred in 11 cases (61%) and HPV was also detected immunohistochemically. Until now six patients had only one recurrence each. Intervals between recurrence were more than 1 year.

In two cases malignant transformations which seemed to be "special" forms of papillomatosis were diagnosed. One case showed a synchronous appearance of

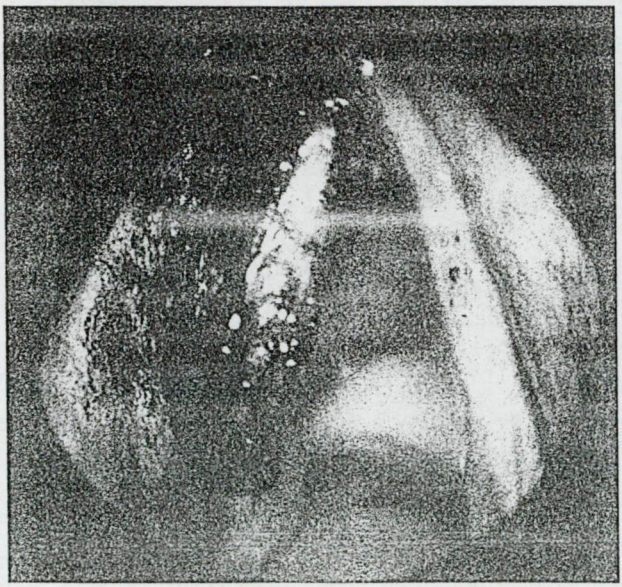

Fig. 2. Adult laryngeal papilloma after CO_2 laser microsurgery.

an adult papilloma on one vocal cord and a cancer on the other vocal cord. The second case was irradiated because of a malignancy and later on an adult papilloma repeatedly appeared on the same vocal cord.

Regarding the development of these two papillomas, no evidence emerged for a real malignant transformation.

Discussion

The classification of papillomatosis was based on the detection of capsid protein of HPV mainly in juvenile onset papilloma, but also in most of the adult papillomas. The juvenile type was differentiated by presence of koilocytes.

In the literature, the PCR method is stated to be a successful method for virus detection [20,21], but it was not available for this study.

The advantage of the described endolaryngeal CO_2 laser microsurgery of papillomatosis is a modern, minimally invasive technique, which provided accurate removal of the papilloma tissue, with a longer recurrence interval. As a minor complication there were only a few postoperative perifocal edemas. The CO_2 laser surgery has become a safe and fast method for removal of laryngeal papilloma, but this surgical intervention is not able to prevent recurrences and seems to be only a symptomatic treatment.

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References

1. Strome M. Analysis of an autogenous vaccine in the treatment of juvenile papillomatosis of the larynx. Laryngoscope 1969;79:272–279.
2. Smith HG, Healy GB, Vaughan CW, Strong MS. Topical chemotherapy of recurrent respiratory papillomatosis. A preliminary report. Ann Otol Rhinol Laryngol 1980;89:472–478.
3. Czigner J, Deutsch O. Endolaryngeal microsurgery in the treatment of adult papillomas. Fül Orr Gégegyógy 1971;17:171–176.
4. Kleinsassser O. Mikrolaryngoscopie und Endolaryngeale Mikrochirurgie. Technik und Typische Befunde. Stuttgart-New York: F.K. Schattauer-Verlag, 1968.
5. Miehlke A, Chilla R, Vollrath M. Cryosurgery and laser surgery in the treatment of malignant and benign laryngeal processes. J Otolaryngol 1979;41:273–278.
6. Brophy JW, Scully PA, Stratton CJ. Argon laser use in papillomas of the larynx. Laryngoscope 1982;92:1164–1167.
7. Czigner J. Gégepapilloma (Laryngeal papilloma), Proceedings of 33rd Congress of Hungarian Otorhinolaryngologists, Szeged, Referatum, 1989;94–96.
8. Strong MS, Vaughan CW, Cooperbrand SR, Healy GB, Clemente MA. Recurrent respiratory papillomatosis: management with the CO_2-laser. Ann Otol Rhinol Laryngol 1976;85:508–516.
9. Bujia J, Feyh J, Kastenbauer E. Photodynamic therapy with derivates from hematoporphyrines for recurrent laryngeal papillomatosis of children. Early results. Ann Otol Rhinol Laryngol Ibero Am 1993;20(3):251–259.

10. Lopez Aguado D, Perez Pinero B, Betancor L, Mendez A, Campos Benales E. Acyclovir in treatment of laryngeal papillomatosis. Int J Pediatr Otol Rhinol Laryngol 1991;21(3):269–274.
11. Einhorn S, Stander H. Interferon therapy for neoplastic diseases in men in vitro studies. Adv Exp Med Biol 1978;100:159–174.
12. Cancura W. Long-term observation in juvenile papillomas of the larynx after surgical removal and ultrasound treatment. Otol Rhinol Laryngol 1977;56:133–137.
13. Chireskin DG, Kuznetsov VP, Onufrieva EK, Pritsker AD. Effectiveness of human leucocytes interferon in children with papillomatosis of the larynx, trachea and bronchi. Vestn Otol Rinol Laryngol 1991;5:19–20.
14. Élő J. CO_2 laser and isoprinosine treatment of laryngeal papillomas. Fül Orr Gégegyógy 1988;34:8–12.
15. Gappa M, Freihorst J, Seudenberg J, Vollrath M, von der Hardt H. Juvenile laryngeal papillomatosis – a case report. Pneumologie 1991;45(11):936–938.
16. Morrison GA, Evans JN. Juvenile respiratory papillomatosis: acyclovir reassessed. Int J Pediatr Otol Rhinol Laryngol 1993;26(2):193–197.
17. Di Lorenczo TP, Steinberg BM. Laryngeal keratinocytes show variable inhibition of replication by TGF-B. J Cell Sci 1990;96(1):115–119.
18. Grunert D, Stier B, Klingebiel T, Schoning M. Ultrasound diagnosis of larynx with the aid of computerized sonography. Otol Rhinol Laryngol 1989;68(4):236–238.
19. Arndt O, Zeise K, Bauer J, Brock J. Correlation between chronic hyperplastic laryngitis and infection with human papilloma viruses. HNO 1993;41(3):448–456.
20. Arndt O, Zeise K, Bauer J, Brock J. Detection of human papilloma viruses (HPV) in laryngeal papilloma. An in situ hybridization study. Otol Rhinol Laryngol 1992;71:132–136.
21. Watts SL, Brewer EE, Fry TL. Human papilloma virus DNA types in squamous cell carcinomas of the head and neck. Oral Surg Med Pathol 1991;71(6):701–707.
22. Krenács L, Tiszlavicz L, Krenács T, Boumsell L. Immunohistochemical detection of CD 1a antigen in formalin fixed and paraffin embedded tissue sections with monoclonal antibody 010. J Pathol 1993;171:99–104.

Szent-Györgyi Albert Orvostudományi Egyetem, Fül-Orr-Gégeklinika (igazgató: Czigner Jenő dr.), Központi Klinikai Kémiai Laboratórium** (igazgató: Dux László dr.)*

Tumor marker vizsgálatok gége- és hypopharynxtumoros betegeken

CSANÁDY MIKLÓS DR.*, BABARCZI ERZSE DR.**, CZIGNER JENŐ DR.*

Kulcsszavak: **gége-hypopharynx tumor, monitorozás, szérum tumor markerek.**

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ÖSSZEFOGLALÁS

Szerzők a Szegedi Fül-orr-gégeklinika 52 gége- és hypopharynxtumoros betegének és 10 egészséges control-egyén szérum tumor marker vzisgálatáról számolnak be. Műtét előtt és után vizsgálták a vér tumor markerszint változását immunoassay módszerrel carcinoembryonalis antigen (CEA), szöveti polypeptid (tissue popypeptide antigen (TPA) és laphámrák - squamous cell carcinoma) antigen (SCC) markereknél, összehasonlítva a betegség klinikai lefolyásával. Az irodalomban közölt értékszámítások alapján betegeiknél a CEA szenzitivátsa 38%, a TPA markeré 14,5%-nak bizonyult. Az SCC esetében kevés vizsgálatból a szenzitivitást 12%-nak találták. A betegek kóros és nem kóros prae- és postoperativ CEA és TPA tumor marker értékeit összevetve mindkét csoportnál szignifikáns különbség mutatkozik a postoperativ értékek csökkenésében, valamint a követés során ennek változásából néhány esetben következtetést lehetett levonni a recidívák és metasztazisok megjelenésére. Vizsgálataik szerint több praeoperatíve kóros marker szintű betegen a marker értékek közvetlenül a műtét után csökkentek, s a későbbi megfigyelés során ismételt emelkedésük – retrospektíve értékelve – jelezte a kialakuló metasztázist. Ezekben az esetekben a tumor marker vizsgálat alkalmas lehet a betegek monitorozására, de a markerek alacsony szenzitivitása miatt fej-nyaki tumoroknál használatuk ma még korlátozott.

BEVEZETÉS

A tumor markerek, mint ismeretes a daganatsejtek által termelt olyan biológiai anyagok, melyek a szérumból immunoassay módszerrel igen kis koncentrációban kimutathatók. A tumor marker szint vizsgálatok célja a már kialakult, de klinikailag még nem észrevehető daganatos sejtek jelenlétének korai kimutatása. E célt ideális tumor markerek segítségével lehetne elérni, de ezek szenzitivitása és specificitása csak néhány területen teszi lehetővé a betegek szűrését és a kezelés korábbi megkezdését (pl.: prostata tumorok). A fej-nyak területén folytatott tumor marker vizsgálatok eredményei ellentmondóak. Legtöbb szerző szerint több marker együttes alkalmazásával növelhető a szenzitivitás, mert magas szenzitivitású, specifikus marker nem ismert ezen a területen. A leggyakrabban használt markerek a következők: a CEA (carcinoembryonic/carcinoembryonalis antigen) (1, 4, 5, 7, 8, 10–12) elsősorban a gastro-intestinalis betegségek (daganatok) markere, a

*6725 Szeged, Tisza Lajos krt. 111.

TPA (tissue polypeptide antigen/szöveti polypeptid antigen) (4, 6–8) általános proliferációs marker, a CA-19–9 (carbohydrate antigen) (1, 10) a pancreas betegségeiben és coloncarcinomában kórjelző, a SCC (squamous cell carcinoma antigen/laphámrák antigen) (1, 3, 4, 7–12) specifikus a laphámrákokra, ezenkívül a Sialinsav (N-acetilneuraminic acid), és a Cyfra (cytokin fragment) (3, 12), melyeket szintén laphámrák kimutatására lehet használni.

Anyag, módszer

Klinikánkon 52 gége és hypopharynx tumoros beteg (45 férfi/7 nő) és 10 egészséges egyén szérumát vizsgáltuk immunoassay módszerrel. A vizsgált tumor markerek közül a CEA (carcinoembryonalis antigen) és az SCC (laphámrák antigen) szérum koncentrációját Microparticular Enzyme Immunoassay (Imx Abbott-Meia) módszerével, a TPA (szöveti polypeptid antigen) értékeit Chemiluminescence Immunoassay methódusával mértük a Központi Klinikai Kémiai Laboratóriumban (SZOTE), mindkét esetben immunoassay metodikát alkalmaztunk. Az egyik egy kemilumineszcencián alapuló immunoassay technika (TPA), az úgynevezett LIA-MAT. A reakció során először a műanyag csövek falára felvitt monoklonális ellenanyagokkal hozzuk össze a mintában lévő antigéneket, majd hozzáadva a jelölt, arra a tumormarkerre specifikus másik ellenanyagot tartalmazó hordozót, inkubálás során létrejön az antitest-antigén-antitest komplex, majd a kötésben részt nem vevő antitesteket mosási technikával eltávolítjuk. Úgynevezett indító reagensekkel, illetve katalizátorok segítségével elindítjuk a reakciót. A módszer lényege, hogy a luminescens anyagok lúgos közegben, aktív oxigénnel katalizátor jelenlétében gyors oxidációs reakcióba vihetők. Az oxidációs termék mellett intenzív, monokromatikus fény képződik, amelynek intenzitása arányos a luminescens anyag koncentrációjával, illetve a kötésben részt vevő antigénekkel. A keletkezett monokromatikus fényemissziót fotomultiplier segítségével mérjük, a jelet erősítés után a mérőrendszer, a luminométer dolgozza fel. Az antigén koncentrációja arányos a jelintenzitással.

A másik metodika egy MEIA, azaz mikropartikuláris enzim immunoassay módszer. Ebben az esetben az ellenanyag egy mikropartikuláris részecskéhez kötött. A mintában lévő antigén kötődik ehhez az antitesthez, majd a reagensben lévő jelölt ellenanyaggal lép reakcióba. Ez az ellenanyag alkalikus foszfatázzal jelölt, mely a reakcióhoz adott 4-methy-lumbelliferyl foszfátot átalakítja, és így fluoreszkáló anyag keletkezik. A mintában lévő antigén koncentrációja egyenesen arányos a fluoreszcenciával.

Az általunk vizsgált tumor markerek esetében az irodalomban leírt kóros határértékeket vettük figyelembe, így a CEA-t 4 ng/ml, a TPA-t 95 U/l és az SCC-t 1,5 ng/ml feletti szérum koncentráció esetén tekintettük kórosnak. Ezen határértékeket a CEA markernél az elemzések szerint a dohányzás úgy befolyásolja, hogy a betegek 5%-ánál eléri a kóros határértéket, esetleg meghaladja azt.

A TNM beosztás szerint többségében T3-T4, N1-es stádiumú (táblázat) betegeken totalis laryngectomia, parciális gége-, pharynx resectio és nyaki block dissectio történt a tumor kiterjedésétől, elhelyezkedésétől és a metasztázistól függően. Az 52 beteg közül szövettanilag a daganat 5 rosszul, 9 közepesen és 38 jól differenciált carcinomának bizonyult.

Vizsgáltuk a prae- és postoperatív (4–6 héttel a műtét után), valamint 3 és 6 hónap utáni tumor marker értékeket a CEA, TPA és a SCC tumor markereknél. A műtét előtti vérmintából meghatározott tumor marker értékeket összehasonlítottuk a műtét után vett értékekkel, ezen különbségekből szignifikancia szintet számoltunk, valamint a betegek sorsának követése során a tumor marker szint változásokat vetettük össze a betegség klinikai képével.

Ellenőrző csoportként vizsgáltuk 10 olyan felnőtt szérumának tumor marker értékeit is, akik tonsillitis chronica (7 beteg) és deviatio septi nasi (3 beteg) diagnózissal kerültek felvételre és mű-

tétre, anamnezisükben daganatos betegség nem szerepelt, majd ezeket összevetettük a gége/hypopharynxtumoros betegek marker értékeivel.

Betegeink kivétel nélkül dohányoznak, ezért a dohányzás tumor marker szint emelő hatását nem tudtuk vizsgálni.

Eredmények

A vizsgált 10 benignus megbetegedés miatt kezelt beteg vérmintájában az összes tumor marker érték alatta maradt a kóros határértéknek, kivéve egy SCC értéket. A legtöbb CEA érték alatta maradt az 1 ng/ml-es, a TPA az 50 U/l-es és az SCC az 1 ng/ml-es határértéknek is.

A műtét előtti 52 CEA értékből a vizsgálat 20 esetben mutatott kóros szint feletti értéket (4 ng/ml feletti). A TPA esetében 49 preoperatív adatunkból 7 esetben láttunk kóros értéket (95 U/l feletti). A CEA marker szenzitivitása így 38%-nak, a TPA-é 14,5%-nak bizonyult. Ezen tumor markerek specificitása a gége/hypopharynx tumoroknál 90% felettinek bizonyult. Az SCC esetében 17 preoperatív eredményből csak 2 bizonyult a kóros határérték felettinek, így szenzitivitása alacsony (9). Ennek ellenére a mért postoperatív adatok alapján több betegen jelentkezett emelkedett SCC szint metasztázis manifesztálódása mellett (3 esetben) és kialakult metasztázis klinikai jele nélkül (2 eset).

A postoperatív CEA értékek közül (n = 25, a többi beteg nem jelent meg a vizsgálaton) a korábban kórosan emelkedettek is nagy fokban csökkentek, de ezekből 5 nem csökkent a kóros szint (4 ng/ml) alá. Egy esetben nem változott az értéke műtét után. A CEA értékek az esetek döntő többségében csökkentek a preoperatív értékekhez képest, mely szignifikánsnak bizonyult ($p = 0,009$). A postoperatív TPA értékek (n = 24, egy betegnél nem történt vizsgálat) közül az összes korábban kóros érték a 95 U/l-es határérték alá süllyedt és a postoperatív értékek szintén szignifikáns különbséget mutattak ($p = 0,0185$). A további tumor marker meghatározások a preoperatíve kóros tumor marker szintek műtét utáni csökkenése ellenére több esetben ismételten emelkedtek, melyek retrospective korreláltak a metasztázis kialakulásával (táblázat).

Megbeszélés

A gége és hypopharynx tumoros betegek szérumának vizsgálata tumor markerekkel különböző szerzők tapasztalatai alapján a kívánt szenzitivitási értékekhez képest szerényebb eredményeket hozott. A III-IV-es stádiumú betegek vizsgálata magasabb marker szenzitivitás értékeket (CEA 36%, SCC 25%) mutatott (Pestasides) (8), mint az I-II-es stádiumú betegeké (CEA 27%, SCC 20,4%). Becciolini (1) aTPA marker vizsgálata során a túlélési arányt szignifikánsan magasabbnak találta azokban az esetekben, ahol a marker szint alatta maradt a 85 U/l-es határértéknek. Dreyfuss (3) az SCC esetében 38%, a CEA markernél 27%-os szenzitivitást talált, szintén III-IV-es stádiumú tumoroknál és a CEA esetében összefüggést talált a kóros határérték feletti marker szint és a jelenlévő távoli metasztázis között. A dohányzás is mérsékelt emelkedést okozhat a CEA markernél Laarmann (6) szerint, de ez nem éri el a kóros határértéket, melyet fej-nyak tumoros betegeinek marker szintje csupán 13%-ban lépett túl. Walther (10) hasonlóan alacsony szenzitivitási értékeket tapasztalt az általa vizsgált SCC, CA 19–9, és CEA markereknél (12–15%), és a marker vizsgálatok prognosztikus, valamint monitorozási jelentőségét limitáltnak tartja.

Az általunk vizsgált tumor markerek szenzitivitási értékei az irodalmi adatokkal korrelálnak (CEA 38%, TPA 14,5%, SCC 12%) és betegeink többsége III-IV-es stádiumú. A prae- és postoperatív CEA illetve TPA marker értékek összevetése után betegcsoportunkban kétmintás „t" próba segítségével szignifikáns marker szint csökkenést lehetett kimutatni.

I. táblázat **A tumor marker vizsgálatok eredményei**

			CEA	CEA	CEA	CEA	TPA	TPA	TPA	TPA
tumor localisatio	tnm	szövettan	preop.	postop.	I. kont.	II. kont.	preop.	postop.	I. kont.	II. kont.
supraglott. laryngis	T3N0	cc. epid. corn. jól diff.	13.6	7.14	6.54	7.9	29.36	11.28	17.59	17.6
supraglott.	T4N0	cc. epid. corn. rossz diff.	0.61	0.05		2.12	14.86	17.09	11.9	10.26
hypopharyngis	T3N1	cc. epid. corn. jól diff.	4.22	2.53	22.25				20.47	
supraglott	T2N1	cc. epid. corn. rossz diff.	1.01	0.71			9.81	35.5		
hypopharyngis	T3N1	cc. epid. corn. kp. diff.	11.1	1.53	1.93	1.77	45.91	27.61	26.2	8.22
vallecula, rad ling.	T3N1	cc. epid. corn. jól diff.	2.73	1.24	1.42	0.83	48.44	22.56	14	106.5
glott.	T4N0	cc. epid. corn. gyengén	0.67	0.6	0.17	1.56	14.47	10.47	10.9	7.48
hypopharyngis	T4N0	cc. epid. corn. jól diff.	3	1.77	1.91	2.83	36.09	25.89	62.2	69.1
vallecula, rad ling.	T3N1	cc. epid. corn. jól diff.	1.75	1.24	2.17	2.19	19.04	14.36	25.95	27.8
hypopharyngis	T3N1	cc. epid. corn. jól diff.	1.77	1.88			108.9	29.63		
supra, rad. ling.	T3N1	cc. epid. corn. rossz diff.	3.69	2.23			101.2	47.5		
supraglott.	T2N1	cc. epid. corn. jól diff.	4.64	3.97	5.03		29.82	25.15	18.67	
transglott.	T4N0	cc. epid. corn. jól diff.	2.02	1.22			28.71	32.27		
supraglott.	T3N0	cc. epid. corn. jól diff.	4.13	3.72			26.85	21.14		
hypopharyngis	T3N1	cc. epid. corn. kp. diff.	3.54	2.02			31.9	48.43		
hypopharyngis	T3N0	cc. epid. corn. kp. diff.	1.68	1.32	1.7		21.92	37.36	45.31	
larrynx	T3N0	cc. epid. corn. jól diff.	1.29	1.1	1.88	1	46.48	78.31	34.61	43.61
hypopharyngis	T4N2	cc. epid. corn. jól diff.	2.56	2.13			45.1	23.9		
hypopharyngis	T4N1	cc. epid. corn. jól diff.	1.35	2.12			65.5	33		
hypopharyngis	T4N1	cc. epid. corn. jól diff.	5.87	4.7	3.6		126.4	36.23	51.1	
hypopharyngis	T3N1	cc. epid. corn. kp. diff.	1.07	1			16.8	11.44		
vallecula, supragl.	T4N1	cc. epid. corn. jól diff.	3.12	6.4			21.05	24.8		
supraglott.	T2N0	cc. epid. corn. kp. diff.	16.1	8.6			21.93	17.1		
hypopharyngis	T2N0	cc. epid. corn. jól diff.	3.6	2.7			129.7	76.9		
glott.	T1N0	cc. epid. corn. jól diff.	4.9	4.4			12.67	17.57		
			0.00933	(P)			0.0185	(P)		

A műtét utáni tumor marker értékek mérsékelt csökkenése, változatlansága, esetleg növekedése a praeoperative kórosnak bizonyult markereknél felvetheti a tumor residuum vagy már meglévő mikrometasztázis lehetőségét. A műtét utáni követéses időszakban a rendszeresen (3 havonta) meghatározott tumor marker értékek korábban jelezhetik a metasztázis kialakulását, mint a klinikai észlelés. Az ilyen esetekben más diagnosztikai eljárások igénybevételével (nyaki UH, CT, MRI) korábban lehet diagnosztizálni a metasz-tatikus nyirokcsomókat, és a nyaki block dissectiot is korábban lehet elvégezni (pl.: a v. jugularist meg lehet tartani, módosított radikális nyaki disszekció-mRND), mely a prognózist javítja. Két esetben nem tapasztaltunk változást sem a preoperatív, sem a postoperatív, sem a későbbi értékekben a kialakult metasztázis ellenére. Ennek magyarázata többféle lehet: – kicsi a tumor marker szenzitivitása, – nincs specifikus fej-nyaki marker –, a termelt tumor markert (protein) a vérben valami egyéb anyag, receptor megköti, ezért nem mutatható ki. Az erősen emelkedett markerek esetében a postoperatív marker szint néhány esetben (CEA) nem süllyedt a kóros értékhatár alá, de nagymértékben csökkent (pl. 50%-kal). A nem kóros praeoperatív marker szintek postoperatív értékei is többségükben csökkentek, ezért felmerül annak a lehetősége, hogy a marker értékek csökkenésének százalékos arányát adjuk meg, természetesen továbbra is megkülönböztetett figyelmet szentelve az irodalmi kóros határérték feletti adatoknak.

Vizsgálataink során nem találtunk összefüggést a tumor differenciációja, kiterjedése, stádiuma és a tumor markerszintek között. A vizsgált markerek alacsony szenzitivitása miatt a módszert a gége- és hypopharynxtumoros betegcsoport szűrésére nem tartjuk alkalmasnak, de a praeoperatíve kimutatott kóros határérték feletti esetekben követhető a tumor progressziója, illetve a marker szint emelkedése jelzi a műtét után ismételten kialakuló malignus elváltozás (recidíva) megjelenését. A kitűzött célok eléréséhez a fej-nyaki tumorok területén nagyobb szenzitivitással rendelkező tumor markerek alkalmazása lenne szükséges, melyek a malignus folyamat megbízhatóbb követését tennék lehetővé.

IRODALOM

1. *Beccioli, A., Porciani, S., Lanini, A.:* Prognostic significance of tissue polypeptide antigen (TPA) in head and neck carcinomas. Acta-Oncol. 32, 295–299, (1993). – 2. *Clasen, B., Pere, P., Senekowitsch, R.:* Squamous cell carcinoma associated antigen (SCC) as a tumor marker in initial diagnosis of carcinomas of the head and neck region. Results of a prospective study after 24 months. Laryngo-rhino-otologie. 69, 275–280, (1990). – 3. *Dreyfuss, A. I., Clark, J. R., Andersen J. W.:* Lipid associated sialic acid, squamous cell carcinoma antigen, carcinoembryonic antigen and lactic dehydrogenase levels as tumor markers in squamous cell carcinoma in the head and neck. Cancer. 70, 2499–2503, (1992). – 4. *Fischer, F., Egg, G.:* N-acetylneuraminic acid (sialic acid) as a tumor marker in hed and neck cancers. HNO. 38, 361–363, (1990). – 5. *Gordano, C., Spiga, G., Fedrighini, B.:* A cancer marker study in patients with neoplastic pathology in head and neck area. Acta-Otorhi-nolaryngol-Ital. 13, 137–145, (1993). – 6. *Laarman, D. A., van Kamp, G. J., Balm, A. J.:* Carcinoembryonic antigen and head and neck cancer. Clin-Otolaryngol. 16, 182–186, (1991). – 7. *Mevio, E., Benazzo, M., Galioto, P.* Use of serum markers in the diagnosis and management of laryngeal cancer. Clin-Otolaryngol. 16, 90–92, (1991). – 8. *Pestasides, D., Bourazanis, J., Economides, N.:* Squamous cell carcinoma antigen (SCC), carcinoembryonic antigen (CEA), and tumour associated trypsin inhibitor (TATI) for monitoring head and neck cancer. Int-J-Biol-Markers. 8, 81–87, (1993). – 9. *Tamás L., Sziklai I., Ribári O.:* Marker kérdés a fül-orr-gégészeti onkológiában. Orvosi Hetilap. 138, 1261–1262, (1997). – 10. *Walther, E. K., Dahlmann, N., Gorgulla, H. T.:* Tumor markers in the diagnosis and follow up of head and neck cancer: role of CEA, CA 19–9, CSS, TK and dTTP-ase. Head-Neck-Surg., 15, 230–235, (1993). – 11. *Walther, E. K., Dahlmann, N., Gorgulla, H. T.:* Tumor markers in patients with head-neck carcinomas. Laryngo-rhino-otologie. 69, 271–274, (1990). – 12. *Zoller, J., Fiehn, W., Mende et al.:* The diagnostic value of the tumor markers CEA, CA 19–9, CA 15–3 and SCC for detection of recurrent tumor in patients with tumor of head and neck. Dtsch-Z-Mund-Kiefer-Gesichtschir. 14, 254, (1990).

SUMMARY

Csanády M., E. Babarczy, J. Czigner: Tumour marker examinations in patients with laryngeal- and hypopharyngeal tumours
The authors render account of serum tumour marker examinations done in 52 laryngeal- and hypop-haryngeal tumourous patients

of the E. N. T Clinic of the Med. Univ. Szeged and, of 10 healthy control persons. They examined the change of tumour marker level of blood, before and, after operation with immunoessay method by CEA, TPA and SCC markers, in comparison with the clinical course of the disease.

On the basis of literary value-calculations published, the authors found the CEA sensitivity for 38%, the of TPA marker 14,5% in their patients. In case of SCC, the sensitivity has been found for 12% among few examinations.

In compared with pathological and not pathological pre- and postoperative CEA and TPA tumour marker values of patients, there have been observed in both groups a significant difference in decrease of postoperative values and, in course of follow up of the patients, change of these values, there can be drawn a conclusion in some cases, of appearing of recurrencies or metastases.

According to their examinations, the marker values decreased immediately after operation in more patients, who had pathological high marker values before operation and, in the course of later observation the repeated increase showed the metastasis be getting under way – in a retrospective study.

In these cases, the tumour marker examination can be suitable for monitorizing of patients, but the low sensitivity of markers their usage is still limited nowadays.

ZUSAMMENFASSUNG

M. Csanády, E. Babarczi, J. Czigner: Tumormarker-Untersuchungen an Patienten mit Kehlkopf- und Hypopharynxtumoren

Die Verff. berichten über Tumormarker-Untersuchungen aus den Seren von 52 Patienten mit Kehlkopf- und Hypopharynxtumoren und 10 gesunden Kontrollpersonen. Vor und nach der Operation untersuchten sie die Veränderung des Tumormarker-Niveaus des Blutes mit dem Immunoassay-Verfahren anhand der Marker CEA, TPA und SCC und verglichen sie mit dem klinischen Verlauf der Erkrankung. Aufgrund der in der Literatur publizierten Auswertungen fanden sie bei ihren Patienten eine Sensitivität von 38% für CEA, 14,5% für TPA.

Der Marker SCC hatte bei den wenigen Untersuchungen eine Sensitivität von 12%. Im Vergleich der pathologischen und nicht pathologischen prae- und postoperativen Werte der Marker CEA und TPA zeigte sich bei beiden ein signifikantes Absinken der postoperativen Werte. In einigen Fällen konnte ihre erneute Veränderung bei späteren Kontrolluntersuchungen auf Rezidive oder Metastasen schließen lassen. Nach Aussage dieser Untersuchungen war der praeoperativ pathologische Markerwert sofort nach der Operation gesunken, und sein erneuter Anstieg bei der späteren Überprüfung war – retrospektiv bewertet – ein Hinweis auf eine entstehende Metastase.

In solchen Fällen könnte die Tumormarker-Untersuchung zur Monitorisierung der Erkrankung geeignet sein, wegen ihrer niedrigen Sensitivität sind die Marker jedoch für Tumoren der Kopf- und Halsregion nur beschränkt nutzbar.

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Szent-Györgyi Albert Orvostudományi Egyetem, Fül-Orr-Gégészeti és Fej-Nyaksebészeti Klinika

Endoszkópos lasersebészet lehetőségei a gégebemeneti carcinomák terápiájában*

CZIGNER JENŐ DR., CSANÁDY MIKLÓS., IVÁN LÁSZLÓ DR.

Összefoglalás

A vizsgálat célja: *az endoszkópos CO2 laserexcisio kiterjesztésének lehetősége, mint új eljárás laryngopharyngealis carcinomák kiválasztott beteganyagán.*
Beteganyag és módszer: *retrospectiv vizsgálat a SZAOTE Fül-orr-gégeklinika 10 éves gége- és hypopharynxrákos beteganyagában; 1987 és 1997 között 187 szervmegőrző műtétből 22 (12%) laserműtét történt supraglotticus gégerák miatt és 189 hypopharynxrák miatt végzett műtét közül 5 (3%) volt endoszkópos CO2 laserműtét.*
Eredmények: *A 22 supraglotticus daganatos beteg közül 15 tumormentes a laserexcisio után, 6 betegnél kellett localis recidíva miatt ismételt laserexcisiót vagy salvage műtétet végezni. 1 beteg irrezekábilissá vált metasztázisai miatt. Az 5 hypopharynxtumoros beteg közül laserműtét után 4 esetben jelentkezett recidíva. 22 supraglotticus gégetumoros beteg endolaryngealis CO2 laserkezelése bizonyítja az ilyen betegeken történő transoralis laserexcisio létjogosultságát szelektív beteganyagon. Ugyanakkor hypopharynxtumor esetében ez nem bizonyult megfelelő eljárásnak.*
Következtetés: *transoralis CO2 laser alkalmazásával sok esetben elkerülhető a betegeknek nagy megterhelést jelentő külső gégeműtét, tracheotomia, lerövidül a beteg ápolási ideje és csökken az ápolási költség, viszont a betegkiválasztást igen szoros kritériumokra kell alapozni. A korai hangszalagrákok sebészi therápiájában igen jó eredménnyel használható endoszkópos laserműtét kiterjeszthető a supraglotticus marginális rákok válogatott eseteire, de nem eredményes módszer a szaporodó hypopharynxrákok kezelésében.*

Summary

Endoscopic CO2 laser therapy for the laryngo-pharyngeal marginal carcinomas of the larynx
Czigner, J., László, I., Csanády, M.
Objectives: *The possible use of endoscopic CO2 laser surgery for marginal laryngo-hypopharyngeal carcinomas on selected patients.*
Material and Methods: *A retrospective analysis of two selected patient groups operated on with early supraglottic laryngeal or hypopharyngeal carcinoma at the Clinic of Otorhinolaryngology, Szeged in a period of 10 years (1987-97). Conservation surgery was the treatment in 187 patients with supraglottic cancer of the larynx and 22 (12%) of them were selected for endoscopic CO2 laser surgery. Of the 189 operated patients with hypopharyngeal carcinoma only 5 (3%) were selected for laser surgery.*
Results: *Of the 22 supraglottic tumors 15 have no sign of recurrence; recurrences developed in 6 patients and they underwent repeated leser excision or salvage therapy. 1 patient beacame irresecable for multiple metastases. Four of the 5 hypopharyngeal tumors recurred. Endolaryngeal CO2 laser excision of 22 supraglottic tumors proved to be a reasonable method in these selected cases but the result of laser excision of the 5 hypopharyngeal tumors was poor.*
Conclusion: *For the supraglottic tumors endoscopic CO2 laser surgery promises a prognostically good, tolerable treatment without external operation and tracheotomy in selected cases. CO2 laser excision is not recommmended for the treatment of hypopharyngeal tumors.*

Bevezetés

A hangszalagrákok T1 a és b stádiumában az endolaryngealis CO2 lasersebészet nemzetközileg is mind szélesebb körben elfogadott terápiás eljárás (1, 2, 3, 10). Kiterjedtebb, T2–T3 -as daganatok transoralis lasersebészeti eltávolítása a nemzetközi irodalomban is vitatott. Steiner és mtsai, Rudert és mtsai (9, 10, 11) véleményét figyelembe véve, megfelelő indikáció és tapasztalat alapján szóba jöhet bizonyos lokalizációjú gége- és hypopharynxtumorok esetében a transoralis laserexcisio, az esetekben többségében azonban a külső műtét az elsődlegesen választandó beavatkozás. Davis és mtsai ajánlata (4, 5) alapján a gége supraglotticus daganatainál csak szigorúan válogatott esetekben lehet használni a CO2 lasert kuratív célból, elsősorban az epiglottis suprahyoid tumorainál, ezzel szemben az infrahyoid tumoroknál a praeepiglotticus páholy érintettségét is feltételeznünk kell, ezért a külső műtét a megfelelő eljárás. Az infrahyoid tumorok laseres kezelése után Davis és mtsai további kiegészítő sugárkezelést is javasolnak. Zeitels és mtsai véleménye

* A MOT XXII. Nemzeti Kongresszusán, Budapesten 1997. november 10–12. elhangzott előadás alapján.

alapján (12) a laserexcisio akár sugárterápia után is alkalmazható az említett esetekben. Felhívja a figyelmet arra, hogy az endoszkópos laserbeavatkozás minimálisan invazív és a cost-benefit elv alapján költsége kisebb, mint a külső műtétnek. A nagy beteganyagról beszámoló kieli klinika eredményei alapján (9, 10) a kuratív célú endolaryngealis CO2 laserexcisio megfelelő ablatív eljárás a marginális supraglotticus tumorok kezelésében. Felmérésük alapján a postoperativ aspiratio mértéke kisebb, mint a klasszikus supraglotticus gégerezekció után, ugyanakkor felhívják a figyelmet a műtét során előforduló vérzések lehetőségére is. Nem ajánlják viszont a postoperativ besugárzást a műtéti terület fokozott oedemakészsége miatt.

A hazai irodalomban Bánhidy és Kásler (1) a supraglotticus gégetumorok esetében a prae-epiglotticus páholy érintettsége miatt szintén a külső műtétet ajánlja elsősorban és csak kivételes esetekben végeznek kuratív célú endolaryngealis laserexcisiót. Élő és mtsai az epilarynx tumorainak szelektív esetekben történt sikeres endolaryngealis laserexcisiójáról számoltak be 11 fős beteganyagon (6).

Feldolgozásunk célja annak kimutatása, hogy az endoszkópos lasersebészet milyen arányban alkalmazható, mint új eljárás, a marginális laryngo-pharyngealis carcinomák sebészi therápiájában.

Beteganyag, módszer

A Szegedi Fül-orr-gégeklinika 10 éves (1987 májusától 1997 áprilisáig) gége- és gége-garatrákos beteganyagából 27 CO2 laserrel kezelt marginális supraglotticus és hypopharynxtumor miatt operált betegről számolunk be, akik a klinikánkon gyakran alkalmazott CO2 laser-chordectomiákhoz (143 beteg ugyanezen idő alatt) viszonyítva lényegesen kisebb számú műtétet esznek ki (táblázat). 22 (12%) supraglotticus rezekció és 5 (3%) hypopharynxtumor-excisio történt intratrachealis narkózisban endoszkópos/endolaryngealis úton CO2 laserrel. Ugyanezen 10 éves időszak alatt 165 (88%) supraglotticus gégerezekciót végeztünk külső feltárásból. A hypopharynxtumorok terápiájában néhány esetet kivéve – 184 (97%) betegen laryngectomia, illetve laryngectomiával kombinált parciális pharynxrezekció volt az alkalmazott eljárás.

1987–1997 között végzett kuratív célú endoszkópos CO2 laserműtétek és konvencionális külső műtétek hangszalagrák és gégebemenet környéki carcinomák miatt

Műtétek	1987–1997 CO2 laserműtét	1987–1997 Külső műtét
Hangszalagtumorok – chordectomia	143	–
Supraglotticus tumorok – parciális gégerezekciók	22 (12%)	165 (88%)
Hypopharynxtumorok	5 (3%)	184 (97%)

Az indikáció felállításánál figyelembe vettük az ablatív eltávolítás lehetőségével a gége megtartását, a beteg életkorát, életkörülményeit és természetesen azt is, ha a beteg nem egyezett bele a külső műtétbe.

A gégebemenet sebészetében az epiglottis suprahyoid, szabad széli tumorait kezeltük elsősorban laserrel, de az álhangszalag, az aryepiglotticus redő körülírt, kis kiterjedésű tumorainál is alkalmaztuk kiválasztott betegeken.

A hypopharynx T1-es tumorai és a gége marginalis régiójának magasságában elhelyezkedő daganatok esetében kíséreltük meg a laseres eltávolítást.

Supraglotticus tumoroknál a rezekciót Steiner módszere szerint végeztük: az epiglottis középvonalban történő átvágása után laterálisan vezettük a metszésvonalat a tumor kiterjedésének megfelelően. Az aryepiglotticus redőn elhelyezkedő és a sinus piriformis medialis falára terjedő T1-es, felszínes tumorokat az aryepiglotticus redővel együtt, részleges epiglottectomiával és a sinus piriformis megfelelő részének kimetszésével távolítottuk el. Szükség esetén a műtétet radikális, vagy módosított radikális nyaki block dissectióval egészítettük ki „együlésben".

A beavatkozások során CO2 laser (Tungsram) készüléket alkalmaztunk laryngomicroscopiás úton. A műtéti terület feltárására legtöbbször a Weerda-féle laryngoscop volt a legalkalmasabb. A daganatok eltávolításához 10–15 W teljesítményű lasersugarat használtunk folyamatos üzemmódban.

A rezekciók alkalmával szinte minden esetben tapasztaltunk bizonyos fokú vérzést, amit vagy laserrel koaguláltunk, vagy elektrokauterrel csillapítottunk. A postoperativ időszakban tracheotomiát igénylő számottevő oedema egy esetben sem fordult elő.

A betegek gyors gyógyulása mellett ezen endolaryngealis sebészeti beavatkozással a minimálisan invazív, szervmegtartó szemléletet próbáljuk megvalósítani, az onkológiai alapelvek figyelembevételével.

Eredmények

A 187 operált supraglotticus gégerákos eset közül 22 (12%) betegen az álhangszalagot és/vagy epiglottist érintő tumort találtunk alkalmasnak endoszkópos laserkezelésre, akikből 15 beteg T1N0, 7 beteg pedig T2N0 stádiumba volt besorolható a TNM beosztás szerint. Ezen betegek közül a primer kuratív műtét után 15 (68%) jelenleg is tumormentes. Hét esetben (32%) jelentkezett localis recidíva. Ezek salvage terápiájaként egy betegen a 9 hónap múlva jelentkező tumorrecidívát besugarazták, azóta tumormentes. 3 betegnél a recidíva után (22, 9 és 6 hónap után) ismételt laserexcisio történt, jelenleg tumormentesek. Egy másik betegünk többszörös recidíva miatt végzett laserexcisiók után sem egyezett bele a laryngectomiába, jelenleg már távoli metasztázisai vannak, incurabilis. További 2 betegünk közül, akiknél szintén recidívát észleltünk, egyik horizontális supraglotticus gégerezekció, a másik laryngectomia után tumormentes. A bete-

gek átlagos követési ideje 38,8 hónap volt. Egészében tehát ebben a betegcsoportban 15 beteg egyszeri endoszkópos laserexcisio után, 3 beteg ismételt laserexcisiók után és 3 beteg „salvage" terápia után tumormentes, ami (21/22) 95% -os eredménynek felel meg.

A hypopharynxtumorok esetében (5 betegen) 5 beavatkozás történt laser segítségével 189 operált beteg közül, 2 betegnek T1N0, másik kettőnek T1N1, egynek T2N1 stádiumú volt a tumora. Két esetben tumorrecidíva miatt végeztük a kuratív laserműtétet. Három esetben a primer tumort endolaryngealis laserexcisióval távolítottuk el, külső nyaki feltárásból pedig 1 funkcionális nyaki disszekció (FND) és 2 radikális nyaki disszekció (RND) történt a nyaki metasztázis eltávolítására. Jelenleg a műtétek után 1 beteg tumormentes, a másik 4 esetben localis recidíva alakult ki, közülük 2 esetben laryngo-pharyngectomia történt, egy beteg incurabilissá vált (nem egyezett bele külső műtétbe), egy további beteg fixált regionális metasztázis és nagy kiterjedésű recidíva miatt ugyancsak incurabilisnak bizonyult. Az átlagos követési idő 34 hónap volt.

Következtetések

Az endoszkópos CO2 laseroperációk helyét szerettük volna bemutatni a gégebemenet és gége-garat malignus tumorainak szervmegtartó műtéti módszerei között, a nemzetközi és hazai irodalomból idézett ajánlásokra és állásfoglalásokra adandó válaszként, saját tapasztalatok alapján. Véleményünk szerint a már eddig is elfogadott módszer a hangszalagrák és a szabad széli epiglottistumorok mellett (1, 2, 3, 10, 11, 12) néhány kiválasztott T1, T2-es supraglotticus tumor endoszkópos eltávolítására alternatívája lehet a külső parciális gégerezekciónak. Természetesen a több régióra kiterjedő gége- valamint hypopharynxtumorok döntő többségét továbbra is külső műtét (parciális garat-, gégerezekció és laryngectomia) útján távolítjuk el. A klinikánkon endoszkópos laserműtéttel operált eseteink döntő többségét a T1a-b hangszalagtumorok endolaryngealis úton történő CO2 laser-chordectomiája (2, 3, 8) teszi ki, amit önálló terápiaként, rutinszerűen alkalmazunk jó eredménnyel. Az itt ismertetett tapasztalataink szerint a CO2 laserexcisiót T1, T2-es supraglotticus marginális gégetumorok esetében a külső gégeműtét alternatívájaként alkalmazzuk. Ennek indikálásakor azonban számolnunk kell azzal, hogy az így operált betegek kb. egyharmadának újabb beavatkozásra, kiegészítő terápiára is szüksége lehet.

Mindezek alapján hangsúlyozzuk, hogy az endoszkópos CO2 laseroperációt nem tekintjük rutin műtétnek a supraglotticus gégerák terápiájában; azt csak avatott kézben és olyan centrumokban ajánljuk, ahol megvan a lehetőség és gyakorlat a szükségessé váló további terápiára: a már bevált külső műtétekre és/vagy a sugárterápiára.

A gége supraglotticus, marginális tumorai közül a T1, T2-es, felszínesen elhelyezkedő, körülírt elváltozások alkalmasak lehetnek endoszkópos CO2 laseres eltávolításra, természetesen az ablaszticitás elvének figyelembevételével. Az intra- és postoperativ szövődmények között meg kell azonban említeni a vérzés lehetőségét, melyet sokszor csak elektrokauterrel lehet uralni, valamint az enyhe fokú, konzervatív kezelésre gyorsan gyógyuló postoperativ oedemát.

A szövettani vizsgálatok már a laser-chordectomiáknál is kimutatták a rezekció kritikus területét (elülső comissura) (3), a supraglotticus tumoroknál ez a terület a prae-epiglotticus páholy, mely a infrahyoid tumoroknál döntő jelentőségű (1, 4, 10), ezért több szerző csak a suprahyoid epiglottistumorok laserexcisióját ajánlja és CT-vizsgálatot tart indokoltnak a prae-epiglotticus páholy infiltráltságának eldöntésére (5). Recidíva esetén sugárkezelést alkalmaztunk, amely már több mint egy évtizede jól bevált terápiás kombináció (7). A radioterápia kisfokú oedemát idézett elő, de tracheotomiát emiatt nem kellett végeznünk. Fontos szempont a műtét minimálisan invazív jellege, a kisebb műtéti megterhelés, továbbá az, hogy a betegek gyorsabban tanulnak meg nyelni. Tartós aspiratio nem fordult elő a műtéteink után.

Eredményeink alapján kiválasztott esetekben az endolaryngealis CO2 laserexcisiót alkalmazható eljárásnak tartjuk a supraglotticus marginális gégetumorok esetében, a már említett kritériumokkal.

Az 5 hypopharynxtumoros betegünkön végzett laseres tumorexcisio után kialakult gyakori tumor recidíva miatt az eljárást nem tartjuk biztonságosnak. A laryngoscopos feltárás és az alkalmazott CO2 laser egyenes vonalú terjedése miatt a rezekciós vonalak iránya korlátozott és maghatározott, a tumor mélyebb szöveti terjedésének megítélése is nehezebb (10).

Beteganyagunkon szerzett szerény tapasztalatunk alapján ez az alternatív lehetőség – véleményünk szerint – még kis kiterjedésű hypopharynxtumorokra sem áll fenn. Ezt a véleményünket támasztja alá az a tény, hogy a hypopharynxban elhelyezkedő tumorok szövetrétegi mélységi terjedése az anatómiai viszonyokból következően nem könnyen ítélhető meg, ennél fogva bizonytalan, hogy a tumor laserexcisiója az ép, már daganatsejteket nem tartalmazó szöveti rétegben történik.

Az endolaryngealis laserexcisio mellett manifeszt nyaki metasztázis esetén az együlésben történő nyaki block dissectiót elvégezhetőnek tartjuk.

Az endolaryngealis úton laserrel operált betegeink többségének ápolási ideje néhány nappal rövidült (8), szemben a külső műtétekkel, de természetesen ez nem lehet szempont a külső vagy endolaryngealis műtét indikációjának felállításánál. Klinikánkon a supraglotticus- és hypopharynxtumorok döntő többségét jelenleg is külső műtéttel távolítjuk el. A T3, T4-es gége-hypopharynxtumorok kuratív célú laserexcisióját klinikánkon nem végezzük. A CO2 laserkezelést palliatív céllal is használjuk pl. suffocatio esetében vagy a daganat megkisebbítésre (ún. laser-debulking), illetve biopsia végzésére külső műtétet megelőzően.

Irodalom

1. Bánhidy, F., Kásler, M.: The use of lasers in Otorhinolaryngology and head and neck surgery. Fortschritte der Onkologie 16. Akedemie Verlag. Berlin (1989).
2. Czigner, J., Sávay, L.: Endoscopic CO2 laser surgery for vocal cord cancer. Diagnostic, Therapeutic Endoscopy 1, 69-74 (1994).
3. Csanády, M., Czigner, J., Sávay, L.: Endolaryngeal CO2 Laser Microsurgery of Early Vocal Cord Cancer. A retrospective study. Adv. Otorhinolaryngol. Lasers in Otorhinolaryngol and Head and Neck Surg. Karger, Basel 219-221 (1995).
4. Davis, R. K., Shapsay, S. M., Strong, M. S., Hyams V.J.: Transoral partial supraglottic resection using the CO2 laser. Laryngoscope 93, 429-432 (1983).
5. Davis, R. K., Hayes, J. K.: Management of supraglotic cancer: Selected endoscopic laser resection and postoperative irradiation. 49 Adv. Otorhinolaryngol, Lasers in Otolaryngol and Head and Neck Surg. Karger, Basel 231-236 (1995).
6. Élő, J., Koppány, J., Takácsi-Nagy, L.: A gége marginális tumorainak CO2-laser-sebészete. Fül-orr-gégegyógyászat. 42 (2) 101-106 (1996).
7. Németh, Gy., Bánhidy, F.: Telekobalt besugárzás a gégecarcinóma CO2 laseres kezelése után. Fül-orr-gégegyógy. 31, 176-178 (1985).
8. Rovó, L., Sávay, L., Czigner, J.: Laser surgery as the organ sparing treatment for vocal cord carcinoma. Cost benefit realtion in 100 cases. Radiol. Oncol. 31, 199-201 (1997).
9. Rudert, H., Werner, J. A.: Endoscopische Teilresectionen mit dem CO2 laser bei Larynxkarzinomen. I. Resectionstechniken. Laryngorhinootologie 73, 71-77 (1994).
10. Rudert, H.: Larynx- und Hypopharynxkarzinome. Endoscopische Chirurgie mit dem Laser: Möglichkeiten und Grenzen. Adv. Otorhinolaryngol. suppl. I., 3-18 (1991).
11. Steiner, W.: Experiences in endoscopic laser surgery of malignant tumors of upper aerodigestive tract. Adv. Otorhinolaryngol. 39, 135-144 (1988).
12. Zeitels, S. M., Vaughan, Ch.W., Domanowski, G. F. et al.: Laser epiglottectomy: Endoscopic technique and indications. Otolaryngol Head Neck Surg. 103, 337-343 (1990).

Levelezési cím:
Dr. Czigner Jenő
Fül-Orr-Gégészeti és Fej-Nyaksebészeti Klinika, SZOTE
6725 Szeged, Tisza Lajos krt. 111.

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M. Csanády · L. Iván · J. Czigner

Endoscopic CO_2 laser therapy of selected cases of supraglottic marginal tumors

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Abstract Endoscopic CO_2 laser intervention can be used as conservation surgery for supraglottic laryngeal carcinomas in carefully selected patients. We analyzed retrospectively our experience in managing patients with early supraglottic carcinomas operated on at the Clinic of Otorhinolaryngology, Szeged, Hungary, during the 10-year period between 1987 and 1997. Conservation surgery was the treatment of choice in 187 patients, but only 23 (12%) were selected for endoscopic CO_2 laser surgery. Laser surgery was indicated predominantly for T1 cancer of the epiglottis ($n = 15$), but was also performed for T2 cancers ($n = 8$). Of the 23 supraglottic tumors treated, 16 had no signs of recurrence to date (1.5 to 9 years after surgery) a local control rate of 70%. Six patients with recurrences underwent salvage therapies that included repeated laser excisions ($n = 3$), radiotherapy (to 60 Gy), horizontal supraglottic laryngectomy and total laryngectomy. One patient was not resectable because of multiple metastases. Our experience with endolaryngeal CO_2 laser excision indicates that it is a reasonable method in selected cases of supraglottic tumors, but one-third of the patients required salvage treatment.

Key words Supraglottic cancer · Endoscopic CO_2 laser surgery · Supraglottic laryngectomy

Introduction

Endolaryngeal CO_2 laser excisions of T1a, T1b carcinomas of the vocal cord are now accepted as adequate surgical interventions [1, 2, 4, 11]. Endoscopic removal of T2, T3 laryngeal tumors is controversial. Steiner [12], Rudert and Werner [10, 11] have reported their indications for transoral CO_2 laser excision of supraglottic and hypopharyngeal tumors after careful patient selection. Although their reports represented the surgeon's experience, the better majority of extensive laryngeal and hypopharyngeal carcinomas were found to be resected by external partial laryngectomies and partial pharyngectomies. Davis and Hayes [5] and Davis et al. [6] found that curative CO_2 laser excisions were a good option only in selected early supraglottic cancer patients. This method was satisfactory in tumors of the suprahyoid epiglottis, but the possible infiltration of the pre-epiglottic space by infrahyoid epiglottic tumors were felt to be a requisite for open surgery. Adjuvant radiotherapy was also recommended after resection of an infrahyoid tumor [8]. However, laser excision can be used for epiglottectomy even after radiotherapy, and the method is minimally invasive and cost-effective [9, 13].

Endoscopic CO_2 laser management of supraglottic tumors, especially for tumors of the epiglottis, is an ablative surgical intervention, but laser excision can be useful for palliative reasons or staging purposes. Experiences from the ENT Department in Kiel revealed minimal postoperative aspiration, but did warn about intraoperative bleeding [10, 11]. In the latter reports postoperative irradiation was not recommended because of developing edema at the surgical area.

Bánhidy and Kásler [1] determined that supraglottic tumors were best resected primarily by open surgery because of the possible infiltration of the pre-epiglottic space, and only in exceptional cases could the laser be used for curative purposes. Elsewhere Élô et al. [7] reported 11 selected epilaryngeal cancer patients successfully treated with endolaryngeal laser excision.

Our material was analyzed to show the utility of CO_2 laser surgery as an option in selected cases of supraglottic tumors and the outcome of these endolaryngeal interventions.

Materials and methods

At the Clinic of Otorhinolaryngology and Head and Neck Surgery, Albert Szent Györgyi Medical University, Szeged, Hungary, 23 marginal supraglottic cancer patients underwent curative endoscopic

M. Csanády (✉) · L. Iván · J. Czigner
Albert Szent Györgyi Medical University,
Clinic of Otorhinolaryngology and Head and Neck Surgery,
Tisza Lajos krt. 111, H-6725 Szeged, Hungary

CO_2 laser excisions during the 10-year period between 1987 and 1997. In comparison, 143 patients underwent laser cordectomies. The majority of the supraglottic tumors ($n = 165$) were operated on by open surgery that involved partial supraglottic laryngectomy with neck dissection. Age, social care of the patient, and the patient's non-consent to external surgery also influence the indications for endolaryngeal laser intervention (Figs. 1, 2). Suprahyoid tumors of the epiglottis were treated mostly, but small tumors of the false cord and aryepiglottic fold (Figs. 3, 4) were also resected by laser.

The method described by Rudert and Werner [10, 11] was applied for the tumor resection. In brief, the epiglottis was cut through in the midline and the incision was carried laterally for tumor infiltrations. Tumors of the aryepiglottic fold were removed by excision of the aryepiglottic fold with partial epiglottectomy. Additionally, endolaryngeal excision of tumors was combined with neck dissection when a palpable lymph node was present.

A Tungsram TLS 61 CO_2 laser operating with an output of 10–15 W in a continuous mode was applied for laser interventions and coupled with an Opton microscope. A Weerda laryngoscope provided sufficient exposure of the larynx. All procedures were performed under general anesthesia.

Some bleeding occurred during laser resection, but complete hemostasis was accomplished either with the laser or by electrocauterization for severe bleeding. Some of the patients needed feeding tubes because of postoperative aspiration, but all tubes were removed in a few days after surgery. No patient had significant postoperative edema, so no patient required tracheotomy.

Results

Of the 187 supraglottic cancer patients operated on, only 23 (12%) were treated as endoscopic CO_2 laser resections. This was carried out with neck dissection in one patient. Tumors were located mainly on the epiglottis, but some had also infiltrated the false cord. Fifteen of the patients had T1N0 tumors, 7 patients T2N0 lesions and one patient a T2N1. Sixteen of the patients have had no sign of recurrence, for a local control rate of 70%. Radiotherapy (to 60 Gy) was required in one patient as salvage therapy for recurrent tumor after 9 months. Laser reexcisions were done in three patients. Recurrences were found at 6 months, 9 months and 22 months, with no further evidence of tumor subsequently. A horizontal supraglottic resection was performed in one patient and total laryngectomy as salvage therapy in another patient with no signs of recurrence to date. Repeated laser resections were performed in a patient who refused open surgery, but this patient became incurable because of tumor metastases. The average follow-up time has been 38.8 months. Of the supraglottic cancer patients 16 were free of tumor after a single laser resection, 3 after repeated laser resections and 3 after salvage therapy (Fig. 5).

In our clinic laser cordectomy is now a routine method for the T1a and T1b glottic tumors [2, 4, 9]. This method can be safely used for early tumors of vocal cord [1, 2, 4, 13] and is also applicable to tumors of the suprahyoid epiglottis and highly selected cases of the infrahyoid epiglottis and other T1, T2 supraglottic tumors [11, 12]. Nevertheless, the vast majority of T2, T3, T4 tumors were removed by open surgery in our department.

The limitations of surgery involved our inability to explore the tumor through a laryngoscope after being targeted

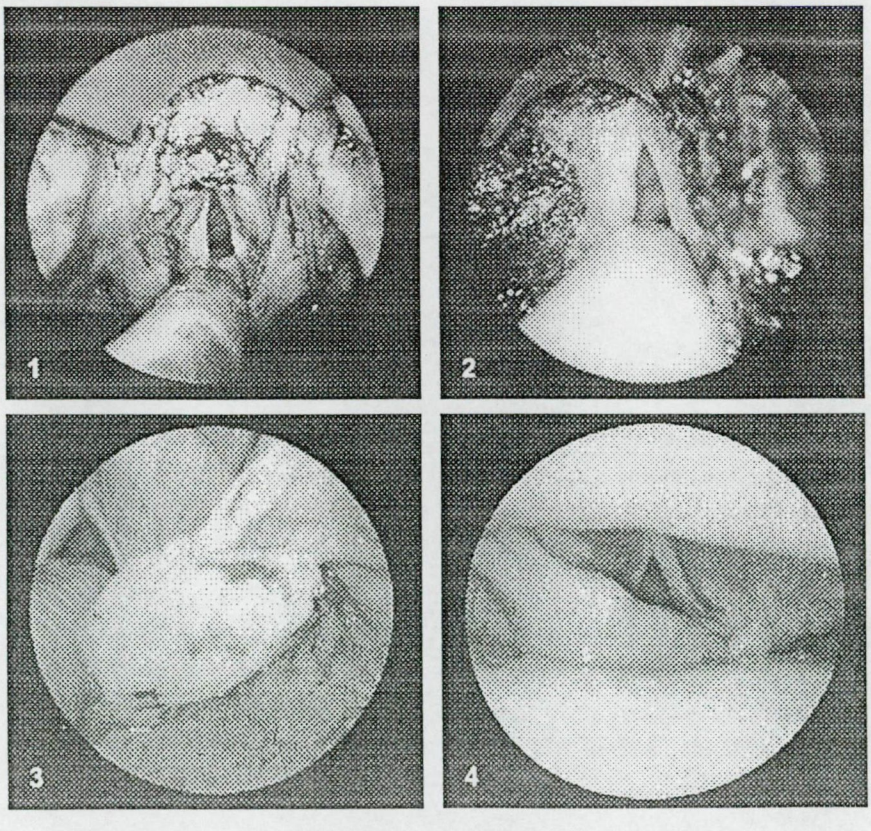

Fig. 1 T2 supraglottic tumor infiltrating the petiole and left false cord

Fig. 2 Postoperative photograph after CO_2 laser treatment of the supraglottic tumor

Fig. 3 T2 tumor of the right aryepiglottic fold

Fig. 4 Postoperative follow-up photograph after CO_2 laser resection of the false cord and aryepiglottic fold

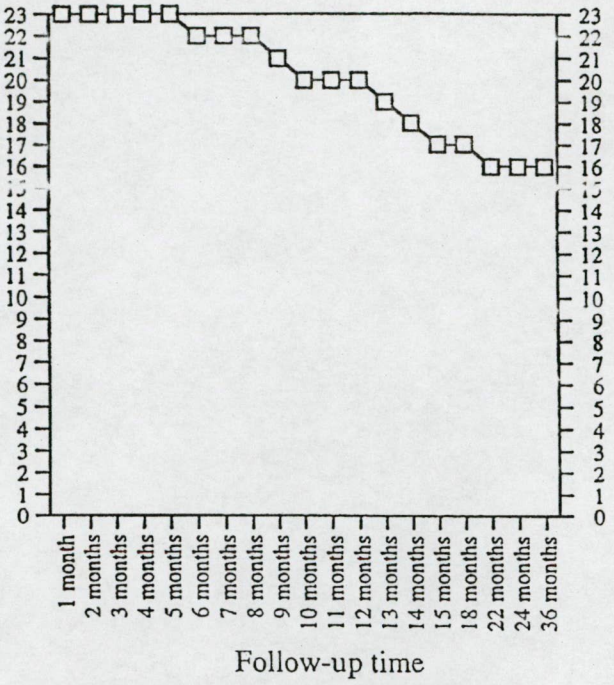

Fig. 5 Three-year disease-free survival of supraglottic cancer patients without local recurrence after endoscopic CO_2 laser excision

by the CO_2 laser. Intra- and postoperative complications involved bleeding that sometimes required electrocauterization for control, aspiration managed by feeding tubes, and a mild laryngeal edema that could be treated by steroid. Postoperative aspiration was not a significant problem in any patient.

The critical area for supraglottic laser resection was the pre-epiglottic space [1, 6, 11], similar to the anterior comissure for laser cordectomy [2]. To detect infiltration of the pre-epiglottic space, CT scan is required [6]. If radiotherapy (30 Gy to 60 Gy) is then used for recurrence after laser resection, no severe complications (such as edema) have been seen to date.

On the basis of our results, endolaryngeal laser surgery is an applicable method for the removal of selected supraglottic cancers. Neck dissection can also be performed together with the endoscopic laser excision if palpable metastases are present. Our study shows that endolaryngeal CO_2 laser surgery serves as an alternative, ablative method to open surgery for selected T1 and T2 supraglottic cancer patients, but one-third of the patients may need salvage therapy. The local recurrence rate after supraglottic laryngectomy at our clinic is under 7% [3]. In our clinic T3 and T4 tumors were not operated on endoscopically, but certainly the CO_2 laser provided a good option for palliative laser debulking of tumors for endoscopic biopsy or to free the airway.

References

1. Bánhidy F, Kásler M (1989) The use of lasers in otorhinolaryngology and head and neck surgery. (Fortschritte der Onkologie 16) Akademie Verlag, Berlin
2. Csanády M, Czigner J, Sávay L (1995) Endolaryngeal CO_2 laser microsurgery of early vocal cord cancer. A retrospective study. (Advances in Otorhinolaryngology Lasers in Otorhinolaryngol and Head and Neck Surgery) Karger, Basel, pp 219–221
3. Czigner J (1993) Über die funktionserhaltende Kehlkopfkrebs-Chirurgie in Ungarn – Ein historischer Rückblick und 25 jährige eigene Erfahrungen. Laryngorhinootol 72:417–420
4. Czigner J, Sávay L (1994) Endoscopic CO2 laser surgery for vocal cord cancer. Diagn Ther Endosc 1:69–74
5. Davis RK, Hayes JK (1995) Management of supraglottic cancer: selected endoscopic laser resection and postoperative irradiation. (49 Advances in Otorhinolaryngology Lasers in Otolaryngol and Head and Neck Surgery) Karger, Basel, pp 231–236
6. Davis RK, Shapsay SM, Strong MS, Hyams VJ (1983) Transoral partial supraglottic resection using the CO_2 laser. Laryngoscope 93:429–432
7. Élô J, Koppány J, Takácsi-Nagy L (1996) CO_2 laser surgery of the marginal laryngeal tumours. Hung J Otorhinolaryngol 42: 101–106
8. Németh Gy, Bánhídy F (1985) Telecobalt radiotherapy after CO2 laser treatment. Hung J Otorhinolaryngol 31:176–178
9. Rovó L, Sávay L, Czigner J (1997) Laser surgery as the organ sparing treatment for vocal cord carcinoma. Cost benefit relation in 100 cases. Radiol Oncol 31:199–201
10. Rudert H, Werner JA (1994) Endoskopische Teilresectionen mit dem CO2 laser bei Larynxkarzinomen. I. Resektionstechniken. Laryngorhinootologie 73:71–77
11. Rudert H, Werner JA (1995) Endoscopic resections of glottic and supraglottic carcinomas with CO_2 laser. Eur Arch Otorhinolaryngol 252:146–148
12. Steiner W (1988) Experiences in endoscopic laser surgery of malignant tumours of upper aerodigestive tract. Adv Otorhinolaryngol 39:135–144
13. Zeitels SM, Vaughan ChW, Domanowski GF, Fullihan NS, Simpson GT II (1990) Laser epiglottectomy: endoscopic technique and indications. Otolaryngol Head Neck Surg 103:337–343

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Miklós Csanady · László Rovó · József Jóri

Combined use of endoscopic CO_2 laser excision of a marginal laryngeal tumor, radical neck dissection, and perioperative laterofixation of the opposite vocal cord

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Abstract We report the use of endoscopic laser excision of a marginal laryngeal tumor, radical neck dissection, and laterofixation of a paralyzed vocal cord in a 66-year-old man who had an early-stage right supraglottic endolaryngeal tumor and ipsilateral neck metastasis. He had a left vocal cord paralysis after a left pneumonectomy that was performed 5 years previously. The primary laryngeal tumor was excised by endoscopic CO_2 laser resection, and a simultaneous radical neck dissection was carried out. Postoperatively, severe inspiratory dyspnea developed because of the surgical intervention on the right side causing moderate laryngeal edema and limited movement of the right vocal cord in addition to the paralyzed left side. An endolaryngeal laterofixation of the paralyzed left vocal cord was performed to provide the patient with an adequate airway instead of tracheostomy. This patient had a 2 years' follow-up without recurrence of tumor. In the meantime movement of the right vocal cord has returned, so that the patient's voice was socially acceptable and he has a functioning larynx.

Key words Endolaryngeal tumor · Neck metastasis · CO_2 laser excision · Neck dissection · Vocal cord laterofixation

Introduction

Minimally invasive surgery can have an important role in the management of laryngeal and pharyngeal tumors, especially in stage T1 and T2 disease. Both the endoscopic excision of the tumor with the CO_2 laser and endoscopic laterofixation of the vocal cord are useful, minimally invasive methods with a reasonable indication. The CO_2 laser is generally accepted for early T1a, T1b vocal cord tumors [2–4, 9] in selected cases can be used for marginal supraglottic tumors [5, 9, 11]. However, only a few reports recommend the endoscopic removal of small laryngopharyngeal tumors [9, 10]. In very selected patients the role of operative time and the lack of consent to partial resection of the larynx and temporary tracheostomy or laryngectomy can be taken into consideration. In such cases endoscopic removal of tumor can be suggested. The critical point of this surgical intervention is the ablative removal of tumor and the limited cutting plane of the CO_2 laser, so that an intraoperative frozen section is needed for pathological examination to determine tumor-free margins. A radical neck dissection is a standard method for the removal of lymph node metastases and can be performed in combination with endolaryngeal excisions of tumor [1].

Bilateral vocal cord palsies previously necessitated a tracheostomy but at present can be avoided by endoscopic methods with reasonable results such as laser arytenoidectomy, cordectomy, and laterofixation of the vocal cord [6, 7]. Lichtenberger [8] reported a helpful technique for widening the glottis in which the vocal cord can be sutured with an endo-extralaryngeal needle carrier and pulled laterally. This method also provides a steady laterofixation of the vocal cord and a better airway for the patient that can be checked by spirometry [6, 7]. There has been no need for tracheostomy for bilateral vocal cord palsies in our Department since we began to use this method.

Case report

A 66-year-old man with a T1 tumor on his right aryepiglottic fold (Fig. 1) was admitted to our clinic after complaining of dysphagia. In addition to the tumor, a palpable ipsilateral lymph node metastasis (N1) was found. The patient had required removal of his left lung as treatment for a bronchial carcinoma 5 years previously, with surgery complicated by a left vocal cord palsy. Sonography of the neck with needle aspiration was performed showed a carcinoma metastasis in the neck. The unilateral vocal cord palsy made him hoarse, but no abnormality in his breathing was found. The patient did not a consent to an external operation, but agreed with endola-

M. Csanády (✉) · L. Rovó · J. Jóri
Department of Otorhinolaryngology, Head and Neck Surgery,
Albert Szent-Györgyi Medical University,
6725 Tisza Lajos krt. 111, Szeged, Hungary
e-mail: miki@orl.szote.u_szeded.hu,
Tel./Fax: +36-62-310141

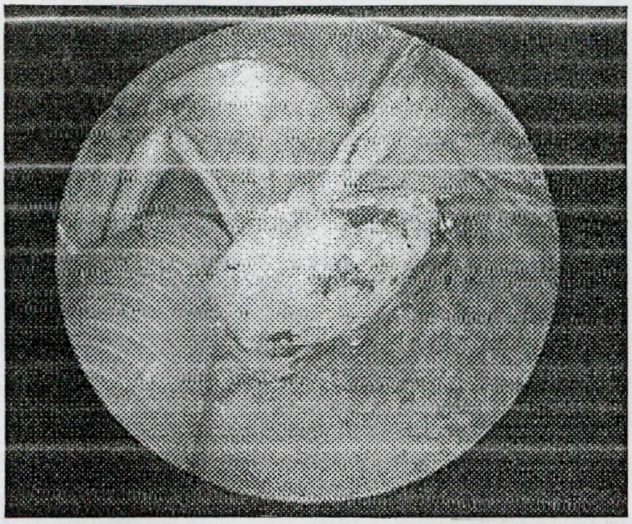

Fig. 1 Preoperative endoscopic photograph of the tumor on the right aryepiglottic fold. The paralyzed left vocal cord has been pushed laterally by the tube

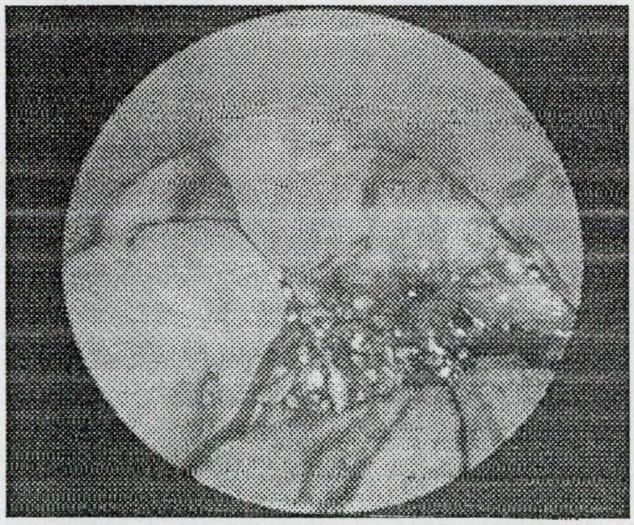

Fig. 2 Postoperative endoscopic photograph of the larynx after CO_2 laser excision of tumor

ryngeal CO_2 laser excision of the tumor and radical neck dissection. Radiotherapy of the primary lesion was also considered, but CO_2 laser excision and radical neck dissection were decided upon because of size of the metastasis and the foreseeable shorter hospitalization of the patient. This was also supported by our more than 10 years' experience with endoscopic CO_2 laser treatment of laryngeal tumors [2, 3] and the published results in the literature [9, 10].

Under general anesthesia endoscopic laser excision of the tumor of the aryepiglottic fold and right radical neck dissection were performed but without tracheostomy. Histological examination revealed tumor-free margins of the specimen of the aryepiglottic fold and a carcinoma metastasis in the neck that was 3 cm in diameter. Two lymph nodes were present that observed infiltration of the capsule of the nodes but no extracapsular spread. There was a slight dyspnea after surgery, and therefore steroid and calcium injections were administered. On the second postoperative day severe dyspnea developed because of limited movement of the right vocal cord (with a 1-mm-wide glottis) and edema. The forced inspiratory volume (FIV_1) was 0.85 l. Two alternatives were considered for improving the airway: tracheostomy or endoscopic laterofixation of the vocal cord.

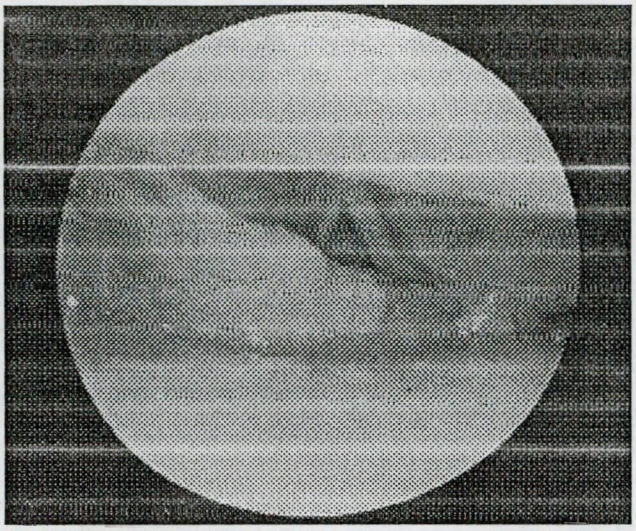

Fig. 3 Endoscopic view of the healed right aryepiglottic fold and wide glottis at 6-month follow-up. The left vocal cord is laterofixed in abduction

Laterofixation was performed on the paralyzed left vocal cord. Two endo-extralaryngeal sutures were inserted endoscopically with the Lichtenberger [8] needle carrier to the posterior-third of the left vocal cord and tied under the skin and over the prelaryngeal muscle. Postoperatively, the glottis width was shown to be 3.5 mm and to provide a sufficient airway without tracheostomy (FIV_1 = 2.25 l). Slight aspiration was observed after the surgical interventions for 3 days, but later there was no further complication.

The patient's voice postoperatively became weaker and "breathy." Later its quality significantly increased proportionally to the recovery of the contralateral vocal cord. The endolaryngeal site of the laser excision was covered by fibrin for 4 weeks (Fig. 2) and the skin incision of the neck dissection healed in 8 days. Postoperative radiotherapy of the neck was not given because of the pathological finding showing tumor-free margins of the primary tumor specimen and only two metastatic lymph nodes without extracapsular spread, allowing us to follow a wait-and-see policy. The patient was checked in following every 2 months and now has been followed for 2 years without locoregional recurrence (Fig. 3). Movement of the right vocal cord has gradually returned.

Discussion

Endolaryngeal CO_2 laser excision of laryngeal marginal (T1) tumors can be performed in selected patients, but the extended use of this method is controversial [5]. The combined utilization of endolaryngeal excision of primary tumor with radical neck dissection can also be an ablative method for the removal of tumor [1], which leaves laryngeal functions almost intact. The described indication of this surgical intervention is limited but in some selected cases can be useful. Radiotherapy of the primary tumor and postoperative neck can also be indicated, but CO_2 laser treatment for T1 marginal tumors of the larynx can result

in as high a rate of successful cure as radiotherapy. Since a patient's hospitalization is shorter, our opinion was that it was an indicated therapy of choice in our case.

The endolaryngeal edema developing postoperatively and near bilateral vocal cord adductor positioning necessitated laterofixation of the previously paralyzed vocal cord. The glottis became wide enough to discharge the patient. The postoperative follow-up time is now 2 years without locoregional recurrence, and the patient still has the endo-extralaryngeal cord sutures in place. In the meantime the mobility of the right vocal cord has gradually returned to provide an acceptable voice.

In highly selected cases the surgical treatment presented gives the opportunity of minimally invasive surgery in the laryngopharynx and a better postoperative quality of life. This case report with the combination of the surgical procedures used also represents a "save the life and save the larynx" therapeutic policy.

References

1. Ambrosch P, Freundenberg L, Kron M, Steiner W (1996) Selective neck dissection in the management of squamous cell carcinoma of the upper digestive tract. Eur Arch Otorhinolaryngol 253:329–335
2. Bánhidy F, Kásler M (1989) The use of lasers in otorhinolaryngology and head and neck surgery Fortschritte der Onkologie 16. Akademie, Berlin
3. Csanády M, Czigner J, Sávay L (1995) Endolaryngeal CO_2 laser microsurgery of early vocal cord cancer. A retrospective study. Advances in Otorhinolaryngology. Lasers in Otolaryngol Head Neck Surgery. Karger, Basel, pp 219–221
4. Czigner J, Sávay L (1994) Endoscopic CO_2 laser surgery for vocal cord cancer. Diagn Ther Endosc 1:69–74
5. Davis RK, Kelly SM (1991) Supraglottic resection with the CO_2 laser. Laryngoscope 101:680–683
6. Ejnell H, Mansson I, Hallen O, Bake B, Stenborg R, Lindström J (1984) A simple operation for bilateral vocal cord paralysis. Laryngoscope 94:954–958
7. Jóri J, Rovó L, Czigner J (1998) Vocal cord laterofixation as early treatment of acute bilateral abductor paralysis after thyroid surgery. Eur Arch Otorhinolaryngol 255:375–378
8. Lichtenberger G (1983) Endo-extralaryngeal needle carrier instrument. Laryngoscope 93:1348–1350
9. Rudert H (1991) Larynx- und Hypopharynxkarzinome. Endoscopische Chirurgie mit dem Laser: Möglichkeiten und Grenzen. Adv Otorhinolaryngol [Suppl] 1:3–18
10. Steiner W (1988) Experiences in endoscopic laser surgery of malignant tumors of upper aerodigestive tract. Adv Otorhinolaryngol 39:135–144
11. Zeitels SM, Vaughan CW, Domanowski GF, Fullihan NS, Simpson GT (1990) Laser epiglottectomy: endoscopic technique and indications. Otolaryngol Head Neck Surg 103:337–343

Supraglottic laryngectomy at the end of 20th century. Analysis of 200 cases with supraglottic surgery

Laryngektomia nadgłośniowa w końcu XX wieku, analiza materiału 200 przypadków

J. Czigner, M. Csanády

Clinic of Otolaryngology & Head and Neck Surgery, University of Szeged, Hungary

INTRODUCTION

The majority of patients with supraglottic cancer of the larynx are treated with radiotherapy in West Europe [10, 12, 14]. If primary surgery is advocated [7, 11, 9], total laryngectomy has been applied dominantly.

On the other hand, supraglottic laryngectomy – with or whitout adjuvant radiotherapy (RT) – has been advocated by others [2, 8, 13] with claims of superior results when compared with radiotherapy alone, especially for T2 and T3 lesions.

Steiner [2], Rudert and Werner [10, 11] have reported their indications for transoral C02 laser excision of supra glottic tumors after careful patient selection. Although the better majority of extensive laryngeal carcinomas were found to be resected by external partial laryngectomies. Davis and Hayes [5] and Davis et al. [6] found that curative C02 laser excisions were a good option only in selected early supraglottic cancer patients. This method was satisfactory in tumors of the suprahyoid epiglottis, but the possible infiltration of the preepiglottic space by infrahyoid epiglottic tumors were felt to be a requisite for open surgery. Laser excision can be used for epiglottectomy even after radiotherapy [8].

Endoscopic C02 laser management of supraglottic tumors, especially for tumors of the epiglottis, is an ablative surgical intervention with minimal postoperative aspiration, but did warn about intraoperative bleeding [10, 11]. In the latter reports postoperative irradiation was not recommended because of developing edema at the surgical area.

Bánhidy and Kásler [1] determined that supraglottic tumors were best resected primarily by open surgery because of the possible infiltration of the pre-epiglottic space, and only in exceptional cases could the laser be used for curative purposes.

MATERIALS AND METHODS

At the Clinic of Otorhinolaryngology of Head and Neck Surgery, University of Szeged, Hungary, of the 204 patients operated on by conservation supraglottic surgery 29 marginal supraglottic cancer patients underwent curative endoscopic C02 laser excisions during the period between 1987 and 1998. The majority of the supraglottic tumors (n=175) were operated on by open surgery that partial supraglottic laryngectomy with neck dissection at the same time.

Group I. Endoscopic supraglottic C02 laser surgery the method described by Rudert and Werner [10, 11] was aplied for the tumor resection. In brief, the epiglottis was cut through in the midline and the incision was carried laterally for tumor infiltrations. Suprahyoid tumors of the epiglottis were treated mostly, but small tumors of the false cord and aryepiglottic fold were also resected by laser. Tumors of the aryepiglottic fold were removed by excision of the aryepiglottic fold with partial epiglottectomy. Some bleeding occurred during laser resection, but complete hemostasis was accomplished either with the laser or by electrocauterization for severe bleeding. Some of the patients needed feeding tubes because of postoperative aspiration, but all tubes were removed in a few days after surgery. No patient had significant postoperative edema, so no patient required tracheotomy. Additionally, endolaryngeal excision of tumors was combined with neck dissection when a palpable lymph node was present.

Group II: Of the 204 patients 175 were operated on by open conservation surgery for supraglottic carcinomas. The open **supraglottic surgery techniques** reported earlier [43], were used as follows.

1) **Simplified (standard) supraglottic laryngectomy** was used for T_1 and T_2 lesions on 129 patients (63%).

2) Forty-six patients were operated on by **extended supraglottic laryngectomy;**

2.1 **Supraglottic subtotal laryngectomy** was designed for resection of the entire supraglottic and one true vocal cord with the arytenoid and ipsilateral thyroid ala below the thyo-arytenoid muscles; 22 patient (11%) were treated by this technique.

2.2 **Supraglottic laryngectomy extended to the base of the tongue;** 24 patients (12%) were operated on by this method.

The reconstruction was performed carefully in all three techniques: 1) the base of tongue is transversely dissected, producing two layers to cover the cuted thyroid cartilage by using two heavy U-shape sutures; 2) appropriate suturing of the new vestibulum laryngis; 3) attention should be taken that the **residual larynx is sinking down** and that to reconstruct a functioning larynx, an important factor is a **secure elevation and the fixation** of the rest-larynx up to the base of the tongue.

RESULTS

Group I. Of the 204 supraglottic cancer patients operated on by conservation supraglottic surgery, only 29 (14%) were treated as endoscopic C02 laser resections. This was carried out with neck dissection in one patient. Twenty-one of the patients had T1N0 tumors, 7 patients T2N0 lasions and one patient a T2N1. Twenty of the patients have had no sign of recurrence, for a local control rate of 69%. Radiotherapy (to 60 Gy) was required in three patients as salvage therapy for recurrent tumor after 9 months, with no further evidence of tumor subsequently. A horizontal supraglottic resection was performed in one patient and total laryngectomy as salvage therapy in four patients with no signs of recurrence to date. Repeated laser resections were performed in a patient who refused open surgery, but this patient became incurable because of tumor metastases.

Group II. Postoperative compplications occured in the II group as follows: An aspiration occured practically in all cases but it improved soon in most patients so that, they were delivered at the end of the 1st month postoperatively. Aspiration pneumonia occured in 7.5%, wound break-down occured in one patient and fistula on 3 patients. Because of laryngeal incompetence 3 patients used a tube for drinking, but 2 patients required completion laryngectomy. Local–regional failure occured in 23%. The most common site for failure was the cervical lymphatics. The local--recurrence rate was really acceptable: 6.6%.

CONCLUSIONS

Conservation surgery for supraglottic cancer of the larynx provides an effective therapeutic modality. Technically, most of the supraglottic lesions of intermediate size can be resected with preservation of voice, but many patients are excluded on medical grounds from a supraglottic laryngectomy. Primary RT is an effective alternative for many of these lesions and for this reason RT is preferred dor early lesions in many insitutions.

In our clinic laser is applicable to tumors of the suprahyoid epiglottis and highly selected cases of the infrahyoid epiglottis and other T1, T2 supraglottic tumors. The limitations of this surgery involved our inability to explore the tumor through a laryngoscope after being targeted by the C02 laser. Intra- and postoperative complications involved bleeding that sometimes required electrocauterization for control, aspiration managed by feeding tubes, and a mild laryngeal edema that could be treated by steroid. Postoperative aspiration was not a significant problem in any patient.

Our study shows that endolaryngeal C02 laser surgery serves as an alternative method to open surgery for selected T1 and T2 supraglottic cancer patients, but one-third of the patients may need salvage therapy.

Age, social care of the patient, and the patient's non-consent to external surgery also influence the indications for endolaryngeal laser intervention. Most proponents of aggressive standard treatments of head and neck cancer in the elderly suggest that treatment modifications or alternatives be considered on the basis of medical comorbidity rather than age. In healthy elderly individuals with minimal or well-controlled comorbidities, all standard treatment modalities are not only reasonable but are also preferred. Patients with pulmonary illness or diminished mental capacity that would interfere with

speech and swallowing rehabilitation may be poor candidates for conservation laryngeal surgery [3].

Nevertheless, the vast majority of T2, T3, T4 supraglottic tumors were removed by open surgical methodes in the last years of the 20th century. The local tumor recurrence rate after supraglottic laryngectomy at our clinic was under 7%. In conclusion, our results show that very good local control rates and voice preservation can be achieved at the supraglottic cancer by these larynx-preserving „classic" surgical methods.

REFERENCES

1. Bánhidy F., Kásler M.: The use of lasers in otorhinolaryngology and head and neck surgery. Akademic Verl. Berlin 1989.
2. Bocca E., Pignataro O., Moreschini O.: Supraglottic laryngectomy: 30 years of experience. Ann. Otol. Rhinol. Laryngol. 1983, 92, 14–18.
3. Bumpous J. M.: Treatment of stage III and IV supraglottic carcinoma. Arch. Otolaryngol. Head Neck Surg. 1999, 125, 1402–1404.
4. Czigner J.: Modification of supraglottic resection of the larynx. [In:] Wigand, M.E., W. Steiner, P.M. Stell (eds): Functional partial laryngectomy. Springer 1984, 197–202.
5. Davis R.K., Hayes J.K.: Management of supraglottic cancer: selected endoscopic laser resection and postoperative irradiation. (49 Advances in Otorhinolaringology Lasers in Otolaryngol. and Head and Neck Surgery) Karger, Basel 1995, 231–236.
6. Davis R.K., Shapsay S.M., Strong M.S., Hyams V.J.: Transoral partial supraglottic resection using the C02 laser. Laryngoscope 1983, 93, 429–432.
7. Glanz H., Kimmich T., Eichhorn Th., Kleinsasser O.: Behandlungsergebnisse bei 584 Kehlkopfcarcinomen an der Hals-Nasen-Ohrenklinik der Univesität Marburg. HNO 1989, 37, 1–10.
8. Herranz-González J., Martinez-Vidal J., Gavilán J., Gavilán C.: Supraglottic laryngectomy. Functional and oncologic results. Ann Otol. Rhinol. Laryngol. 1996, 105, 18–22.
9. Kleinsasser O.: Tumoren des Larynx und des Hypopharynx. Thieme, Stuttgart New York 1997, 77–113, 2–25, 147–152.
10. Laccourreye O., Brasnu D., Merite-Drancy A., Cauchois R., Chabardes E., Ménard M., Laccourreye H.: Cricohyoidopexy in selected infrahyoid epiglottic carcinomas presenting with pathological preepiglottic space invasion. Arch. Otolaryngol. Head Neck Surg. 1993, 881–886.
11. Mann W., Beck Chl., Wannenmacher M., Fuchs M.: Überlebensrate und Funktion bei Kehlkopfhorizontal- und Kehlkopftotalresektion. Laryng. Rhinol. Otol. 1986, 65, 282–286.
12. Price L.A., Shaw H.J., Hill T.: Larynx preservation in previously untreated advanced head and neck cancer: final analysis after 12 years follow-up. J. Laryng. Otol. 1993, 107, [illegible]
13. Robbins K.T., Davidson W., Peters L.J., Goepfert H.: Conservation surgery for T2 and T3 carcinomas of the supraglottic larynx. Arch. Otolaryngol. Head Neck Surg. 1988, 114, 421.
14. Robin P.E., Olofsson J.: Tumours of the larynx. [in:] Scott-Brown's Otolaryngology, Vol. 5, Ch. 11, Butterworths, London 1987, 186–234.
15. Rudert H., Werner J.A.: Endoskopische Teilresektionen mit dem C02 laser bei Larynxkarzinomen. I. Resektionstechniken. Laryngorhinootologie 1994, 73, 71–77.
16. Rudert H., Werner J.A.: Endoscopic resections of glottic and supraglottic carcinomas with C02 laser. Eur. Arch. Otorhinolaryngol. 1995, 252, 146–148.
17. Steiner W.: Experiences in endoscopic laser surgery of malignant tumours of upper aerodigestive tract. Adv. Otorhinolaryngol. 1988, 39, 135–144.

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The Fc-region of a new class of intact bispecific antibody mediates activation of accessory cells and NK cells and induces direct phagocytosis of tumour cells

R Zeidler[1], J Mysliwietz[2], M Csánady[1], A Walz[1], I Ziegler[3], B Schmitt[1], B Wollenberg[1] and H Lindhofer[1]

[1]Clinical Cooperation Group 'Bispecific Antibodies' at the Department of Otorhinolaryngology, Ludwig-Maximilians-University, Marchioninistrasse 15, 81377 Munich; [2]Institutes of Molecular Immunology and [3]Clinical Molecular Biology, GSF-National Research Center for Environment and Health, Marchioninistrasse 25, 81377 Munich, Germany

Summary Bispecific antibodies (bsAb) are considered as promising tools for the elimination of disseminated tumour cells in a minimal residual disease situation. The bsAb-mediated recruitment of an immune effector cell in close vicinity of a tumour cell is thought to induce an antitumoural immune response. However, classical bispecific molecules activate only a single class of immune effector cell that may not yield optimal immune responses. We therefore constructed an intact bispecific antibody, BiUII (anti-CD3 × anti-EpCAM), that not only recognizes tumour cells and T lymphocytes with its two binding arms, but also binds and activates Fcγ-receptor positive accessory cells through its Fc-region. We have demonstrated recently that activated accessory cells contribute to the bsAb-induced antitumoural activity. We now analyse this stimulation in more detail and demonstrate here the BiUII-induced upregulation of activation markers like CD83 and CD95 on accessory cells and the induction of neopterin and biopterin synthesis. Experiments with pure cell subpopulations revealed binding of BiUII to CD64+ accessory cells and CD16+ NK cells, but not to CD32+ B lymphocytes. We provide further evidence for the importance of the Fc-region in that this bispecific molecule stimulates Fcγ-R-positive accessory cells to eliminate tumour cells in vitro by direct phagocytosis.

Keywords: phagocytosis; accessory cells; bispecific antibody; tumour

Bispecific antibodies are regarded as efficient tools for the immunological treatment of disseminated tumour cells in minimal residual disease situations. Usually, they are constructed to target tumour cells by a specific or tumour-associated antigen and to recruit one class of immune effector cell, either T cells or accessory cells like monocytes or natural killer cells. However, long-lasting immune reactions in vivo are much more complex and depend on the activation of different classes of immune effector cells, especially in the initial phase of the immune response. This is usually regarded as the major drawback of conventional bsAb that may not yield full immune responses at the tumour site. We have developed a new class of bispecific antibody, that is composed of the two potent subclasses mouse IgG2a × rat IgG2b. BiUII, a member of these new bispecific molecules, targets tumour cells via the pan-carcinoma antigen EpCAM and T-lymphocytes via CD3. But, in contrast to other bispecific molecules described to date (Fanger et al, 1990; Valerius et al, 1997; Weiner et al, 1995), it also binds and activates human Fcγ-receptor-positive accessory cells like monocytes/macrophages, NK cells, and dendritic cells (DCs) via its Fc-region. Activation of these accessory cells results in the upregulation of costimulatory molecules like CD40, CD80, and CD86 and the production of cytokines like IL-2, IL-6, and DC-CK1 (Zeidler et al, 1999).

Although T cells are considered to be the most important cells for tumour cell elimination, they depend on proper antigen presentation by professional antigen-presenting cells (APCs) or activated accessory cells and costimulatory molecules like CD40, LFA-3, CD80, and CD86 in the presence of cytokines such as IL-2 and IL-12 (Inaba and Steinman, 1984; Stüber et al, 1996). This reveals the importance of the subclass combination for induction of activation signals via the Fc-receptor of accessory cells. A similar T-cell redirecting bsAb, SHR-1 (anti-CD3 × anti-CD19), with the subclass combination mouse IgG1 × rat IgG2b was neither able to activate accessory cells via its Fc-region in a clinical study (de Gast et al, 1995) nor in in vitro assays without addition of exogenous IL-2 (Klein et al, 1997). Moreover, the antitumour efficiency of BiUII is strongly reduced when T cells alone are used as effector cells. We therefore postulate that only the activation of more than one class of immune effector cell is necessary to provide optimal antitumour efficiency. Furthermore, phagocytosis, processing, and presentation of tumour material by APCs are prerequisites for the induction of a polyclonal humoral and cellular antitumour immune response. These data are in accordance with the work of Clynes et al (1998), who recently demonstrated the importance of Fc receptors in passive and active immunity to a melanoma model.

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Correspondence to: H Lindhofer

MATERIALS AND METHODS

Cell lines and PBMC preparation

PCI-I (a gift from Dr T Whiteside, Pittsburgh, PA, USA) is an adherent squamous carcinoma cell-line of the head and neck (SCCHN) and is kept in DMEM with 10% FCS. The cell-line expresses EpCAM but lacks CD80 and CD86 as tested by flow

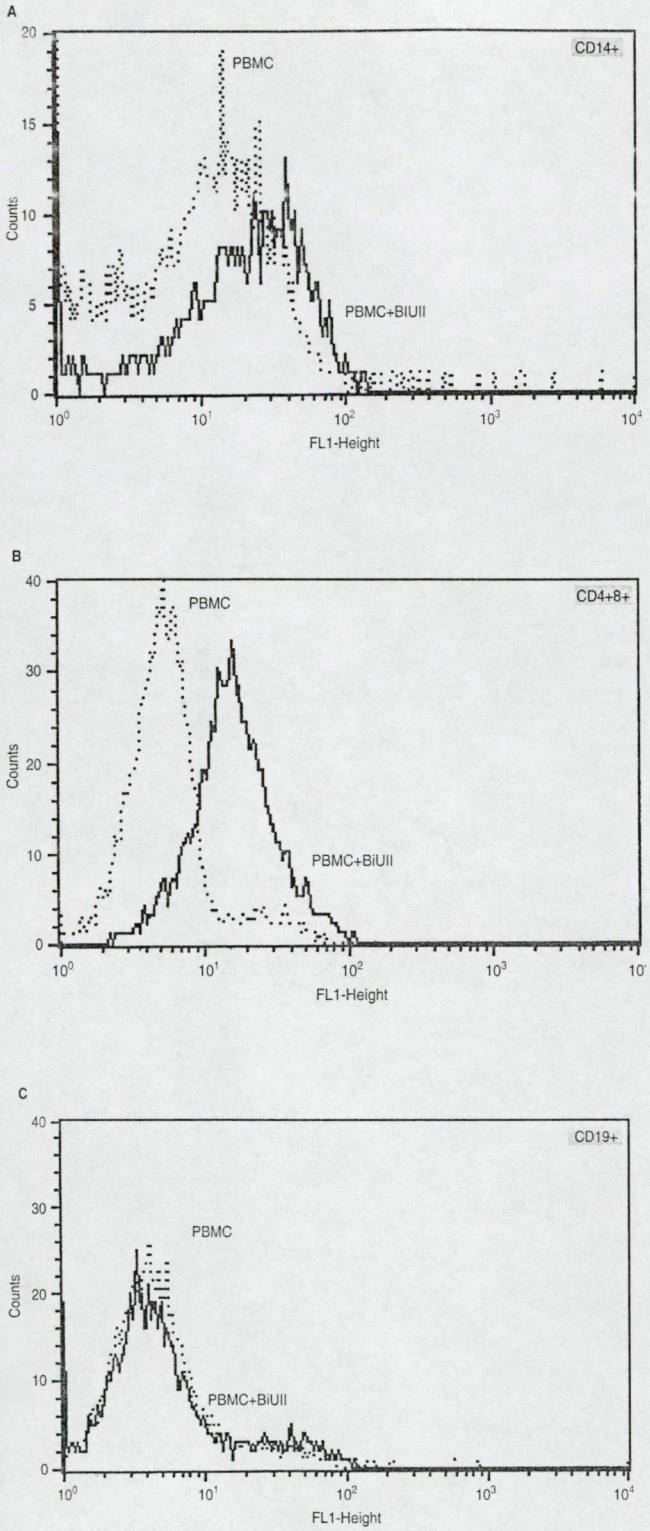


Figure 1 Binding of BiUII to PBMC subpopulations. PBMCs were incubated with BiUII and binding was assessed by FACS analysis. (**A**) BiUII recognizes, albeit weakly, CD14+ monocytes (black line). As monocytes do not express CD3, binding of BiUII is probably mediated by the Fc-region that binds to FcγRI with high affinity. (**B**) BiUII strongly binds to CD3-expressing CD4+/CD8+ T lymphocytes. (**C**) No binding of BiUII antibodies was observed on CD19+ B cells. Isotype control = dotted line.

cytometry (not shown). Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood of voluntary donors by Ficoll density centrifugation.

Monoclonal antibodies

mAbs for FACS analysis were from Pharmingen (Hamburg, Germany) except the DC-specific antibody BMA-X11 (Dianova, Hamburg, Germany).

Generation of dendritic cells

The adherent fraction of PBMCs was incubated for 7 days in Iscove's medium with 5% FCS (both Gibco BRL, Gaithersburg, MD, USA) and 800 U ml^{-1} each of human IL-4 and GM-CSF (both Boehringer Mannheim, Penzberg, Germany).

FACS® analysis

For FACS® analysis, 10^5 cells were incubated with the primary antibody for 30 min on ice in PBS with 2% FCS. Cells were washed twice in PBS and incubated for another 30 min with the second, FITC-labelled, antibody. After two final washings, propidiumiodide was added and flow cytometry was performed using a FACSCalibur® cytometer and the CellQuest analysis program (Becton Dickinson, Heidelberg, Germany). For isolation of highly purified $CD2^+$ cells, PBMCs were incubated with FITC-labeled antibodies and separated on a FACS-Calibur®.

Production of BiUII

The BiUII Quadroma was produced as previously described (Lindhofer et al, 1995). The following hybridomas have been used: 26ll6 (rat IgG2b, anti-CD3, provided by R Schuh, GSF, Germany) and C215 (mouse IgG2a, anti-EpCAM, kindly provided by M Dohlsten, Pharmacia Upjohn, Sweden). To isolate hybrid Ab molecules of the subclass combination rat IgG2b/mouse IgG2a from quadroma, the supernatants were centrifuged, filtered, and loaded onto a 5 ml Econo Pac protein A column (Biorad, Richmond, CA, USA). After washing with 10 volumes of PBS, antibodies with the hybrid heavy-chain configuration were eluted with 0.1 M citric acid, pH 5.1.

Cell culture and killing efficiency

For determination of BiUII-mediated killing of tumour cells and cytokine production, 1×10^4 PCl-1 cells per well (targets = T) were pipetted in 96-well flat-bottom plates (Falcon) and PBMCs or subpopulations of these effectors (= E) were added at E:T ratios from 40:1 to 1:1. BiUII was used at 10 ng per well in a total volume of 100 µl per well RPMI with 10% FCS. Plates were incubated for 3 days at 37°C in a humified atmosphere and 5% CO_2.

Isolation of monocytes/macrophages and NK cells

CD14+ monocytes/macrophages and CD56+/CD3– NK cells were isolated from PBMCs using PE-labelled monoclonal antibodies and a Becton Dickinson FACSVantage cell sorter. Purity of isolated cells was examined by flow cytometry.

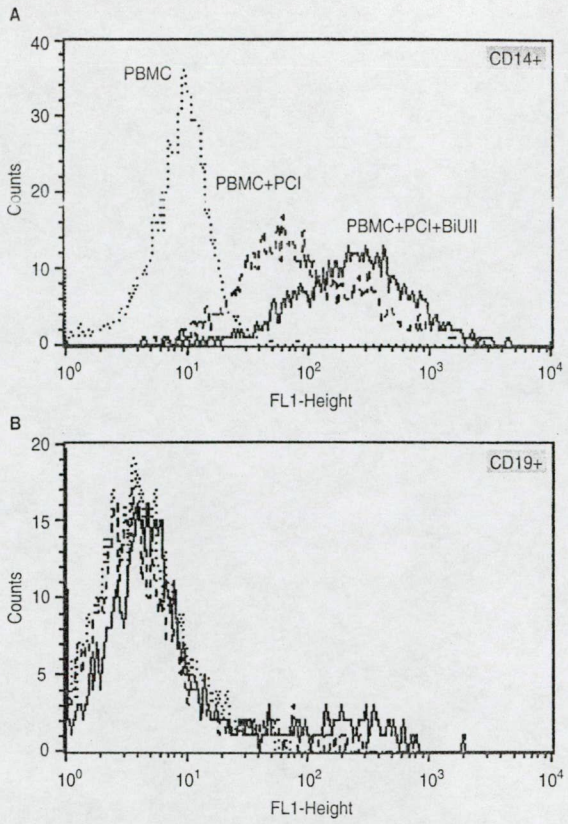


Figure 2 BiUII stimulates accessory cells to phagocytose PCI tumour cells. FITC-labelled PCI cells were cocultivated with PBMCs for 15 h with or without BiUII. After 15 h, monocytes (CD14+) and B cells (CD19+) were analysed for the presence of FITC, indicating the phagocytosis of PCI cells. (**A**) The most prominent FITC mean values (248) were observed in monocytes incubated in the presence of BiUII. A much lower intensity (76) was observed where BiUII was omitted. Background value of PBMC was 9. Similar results were observed after 24 or 48 h cocultivation period (data not shown). (**B**) In contrast, no FITC was incorporated in B cells.

FITC-labelling and uptake of PCI-1 tumour cells

PCI-1 cells were washed twice with Ca^{2+} and Mg^{2+} free PBS. 1 µl of FITC (1 mg ml^{-1} in Ethanol; Sigma, Deisenhofen, Germany) was then added to each 2×10^5 tumour cells in 100 µl PBS, and cells were shaken for 30 min at room temperature. Thereafter, FITC-labelled PCI-1 cells were washed twice with cell culture medium and added to PBMC cultures. The intensity of FITC-labeling was monitored by FACS analysis. Phagocytic capacity of PBMC co-incubated with FITC-labelled PCI-1 cells and BiUII was revealed by FACS after staining with PE-labelled mouse-anti-human-CD14 or –CD19 antibodies. FITC fluorescence intensity of vital CD14+ or CD19+ PBMCs was measured and interpreted as uptake of FITC-labelled PCI-1 tumour cells. Binding of BiUII to PBMC subclasses was revealed by FACS analysis after double-staining with FITC-labelled mouse-anti-rat antibodies (Dianova, Hamburg, Germany) and PE-labelled mouse-anti-human–CD4/CD8, –CD14, or –CD19 antibodies. A combination of gates (vital cells, CD14+ or CD19+ and FCS vs SSC) was used to exclude aggregates of PCI-1 cells with PBMCs from our analysis of phagocytosis of tumour cells.

MTT-Assay

To assess BiUII-mediated tumour cell killing, a colourimetric MTT-based assay was performed as previously described (Heo et al, 1990). Briefly, PCI-1 target cells were plated in wells of a 96-well flat-bottom plate and incubated overnight to prepare semiconfluent cell monolayers. Effector cells were added to the tumour cell monolayers at the appropriate ratios and plates were incubated for 24–48 h. After removing effectors by washing, MTT solution (0.5 mg ml^{-1}; Sigma) was added, and plates were incubated for a further 4 h. The MTT solution was removed and blue crystals of formazan formed in viable tumour cells were dissolved by adding dimethylsulphoxide. Plates were read at 540 nm in a spectrophotometer and results were calculated based on the mean absorbance obtained from at least six wells according to the following formula: % cell death = $100 \times (C–E)/(C–B)$, where C is the optical density reading of the cells with target cells in the absence of effectors (control), B is background without any cell population, and E is the optical density reading of adherent tumour cells remaining in the well after co-incubation with effector cells.

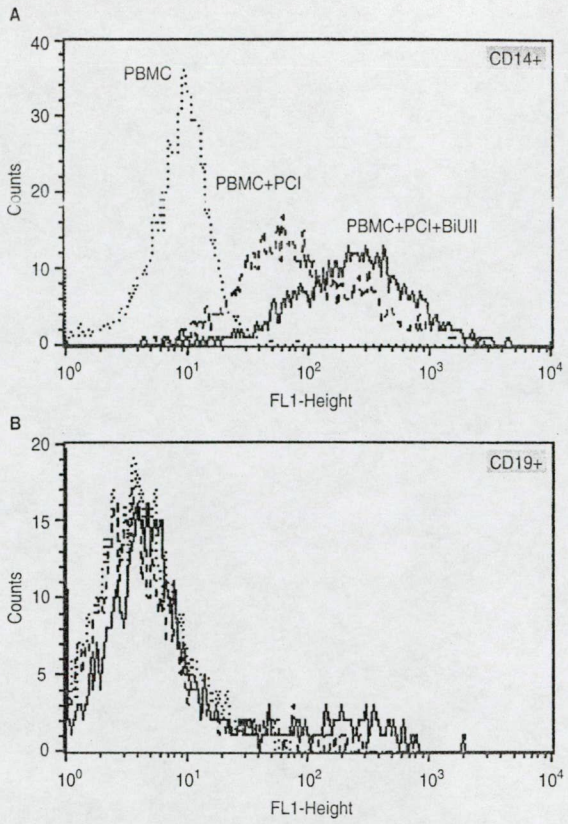
Activity of GTP cyclohydrolase I and cellular pterin levels

The activity of GTP cyclohydrolase I was determined in the supernatant fraction of the cell extracts (Tris/HCl, pH 8.0; 2.5 mM EDTA) after acidic iodine oxidation of the reaction product dihydroneopterin triphosphate. The neopterin phosphates were separated by ionpairing HPLC and fluorometrically detected. Cellular neopterin and biopterin were determined in aliquots of the cell extracts after acidic iodine oxidation, deproteinization by trichloroacetic acid, pre-purification by cation-exchange chromatography and separation by reverse-phase HPLC, basically as described previously (Kerler et al, 1990).

RESULTS

BiUII binds to CD3–, FcγR+ accessory cells

We constructed a new class of bispecific antibody, BiUII, that recognizes epithelial tumour cells via the pan-carcinoma antigen EpCAM (Quak et al, 1990) and redirects T lymphocytes via CD3. We have recently shown that BiUII displays an excellent antitumour activity and also that complete PBMCs are superior to a highly purified T-cell population of the same donor with regard to tumour cell killing (Zeidler et al, 1999). We therefore addressed the question whether BiUII binds peripheral blood monocytes which express the high-affinity Fcγ-RI, CD64 and whether these accessory cells contribute to tumour cell killing. To this end, PBMCs were incubated with BiUII and a FITC-labelled anti-rat Ig antibody and binding was assessed by FACS analysis. As depicted in Figure 1, BiUII binds to CD14+, albeit weakly. Since neither antigen recognized by BiUII (CD3 and EpCAM) is present on monocytes, we concluded that binding of BiUII to Fcγ-R-positive accessory cells is most probably mediated by the Fc region of BiUII. This finding is in agreement with data already published (Haagen et al, 1995). In parallel, we investigated the binding of BiUII to T and B lymphocytes. T lymphocytes express CD3, one



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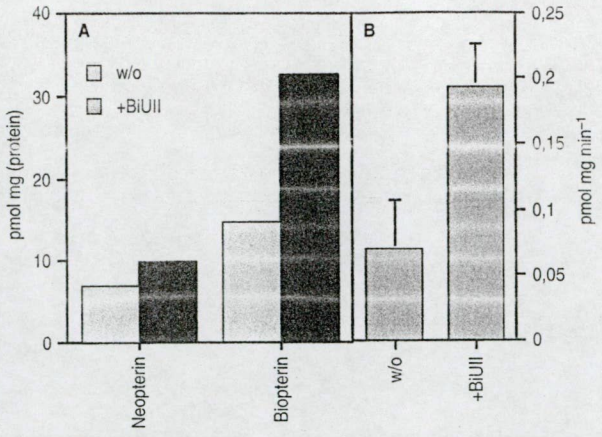

Figure 3 BiUII induces GTP-cyclohydrolase I activity and the production of neopterin and biopterin in monocytes/macrophages. PCI-1 cells were cocultivated with PBMCs either with (100 ng ml^{-1}) or without BiUII for 2 days. (**A**) The production of neopterin and biopterin and (**B**) the activity of GTP cyclohydrolase was determined. BiUII stimulates the production of both biopterin and the monocyte-specific neopterin, as well as the activity of the enzyme. PCI-1 cells per se are negative for all three products.

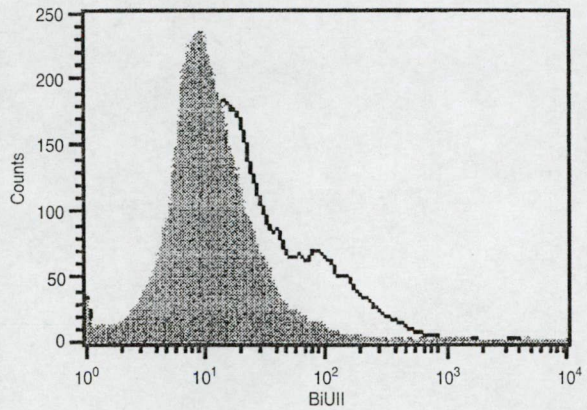

Figure 4 BiUII binds to NK cells. Binding of BiUII to highly purified, CD16+/CD3-NK cells from peripheral blood was investigated by FACS. Mean values are 64 with (black line) and 24 without BiUII (grey trace).

target molecule for BiUII, and consequently a strong binding was observed. In contrast, BiUII does not bind to CD19+ B cells that only express the low-affinity FcγRII, CD32.

BiUII-mediated phagocytosis of PCI-1 cells by CD14+ monocytes/macrophages

Since accessory cells contribute to T-cell activation and tumour-cell elimination in different ways, we wanted to find out whether direct phagocytosis of the tumour cells by CD14+ cells occurs. To this end, PCI-1 tumour cells were stained with FITC and cocultivated for 15 h with PBMCs in the presence of BiUII to assess direct phagocytosis of tumour cells. In control settings, BiUII and/or FITC-labelled tumour cells were omitted. After concultivation, the mean FITC-fluorescence intensity, indicative for the uptake of labeled PCI-1 cells, was measured in vital CD14+ monocytes or CD19+ B lymphocytes. As shown in Figure 2A, uptake of FITC-fluorescence was triggered in CD14+ monocytes/macrophages co-cultivated with BiUII. In contrast, CD19+ B cells from the same donor showed no signs for PCI-1 uptake, even in the presence of the bispecific molecule (Fig. 2B).

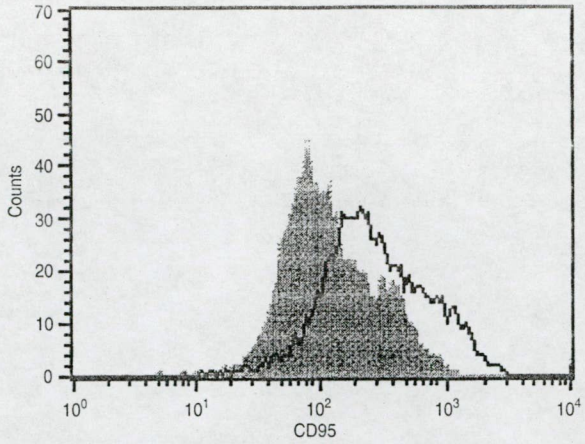

Figure 5 Addition of BiUII to a culture of purified NK cells induces the expression of CD95, an activation marker for NK cells after 1 day of culture. Mean values are 400 (black line) and 182 (grey trace) for NK cells incubated with and without BiUII, respectively.

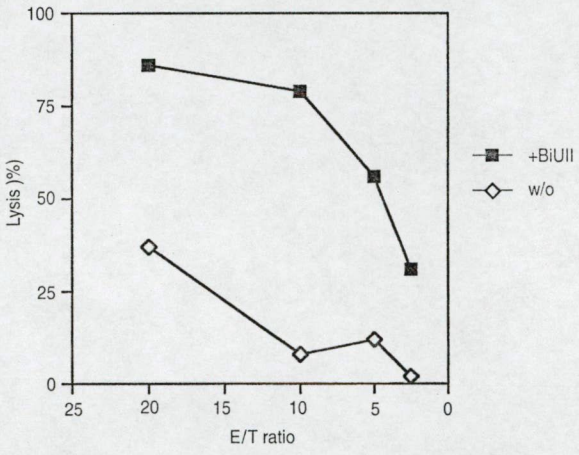

Figure 6 NK cells are stimulated to kill allogeneic tumour cells after incubation with BiUII. Highly purified CD56+CD3−NK cells (effectors) were cocultivated with PCI-1 cells at different E/T ratios for 2 days either with (100 ng ml^{-1}) or without BiUII. Killing of tumour cells (targets) was calculated in a MTT assay as described in Material and methods. One representative of three independent experiments is shown.

BiUII stimulates the production of neopterin and biopterin

Stimulation of T cells causes release of interferon-γ, which in turn induces increased expression of GTP cyclohydrolase I in monocytes/macrophages and in the T cells themselves (Schott et al, 1993; Ziegler, 1990). This enzyme initiates and controls the biopterin synthesis pathway. Therefore, activated T cells produce tetrahydrobiopterin, whereas monocytes/macrophages cannot complete the pathway. They terminate the synthesis pathway after the first step and instead accumulate and shed neopterin. Increase in the activity of GTP cyclohydrolase I and the synthesis of neopterin and biopterin are therefore indicators of monocyte/macrophage and of T-cell activation, respectively (Ziegler, 1990). Figure 3 demonstrates that BiUII induces GTP cyclohydrolase activity and enhancement of neopterin and biopterin production in PBMC after cocultivation with tumour cells in the presence of BiUII, indicative for activation of T cells and monocytes.

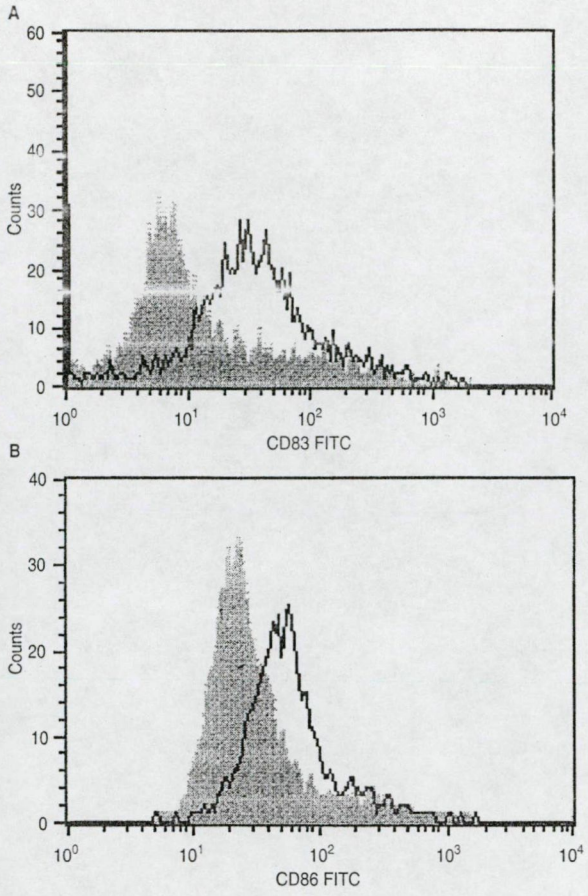

Figure 7 Induction of CD83 and CD86 on dendritic cells. Addition of BiUII for 1 day to a culture of DCs leads to upregulation of (**A**) the activation marker CD83 (**B**) and the costimulatory molecule CD86. Only cells positive for the DC marker BMA-X11 were considered for analysis. With BiUII = black line; without antibody = grey trace.

BiUII activates NK cells to tumour cell lysis

NK cells are known to play a pivotal role for the elimination of tumour cells. Since NK cells express the low-affinity FcγRIII (CD16), we wondered whether BiUII not only binds to CD64+ monocytes/macrophages but also to CD16+ NK cells. We therefore isolated highly purified CD56+/CD3– NK cells and incubated them with BiUII and revealed binding of the bispecific antibody by FACS analysis (Figure 4).

Binding of BiUII to NK cells via CD16 should lead to their activation, resulting in an antitumour activity. We therefore looked for BiUII-mediated induction of CD95 on NK cells, which is recognized as an activation marker for these cells (Medvedev et al, 1997; Robertson et al, 1995) and investigated tumour-cell killing by BiUII-activated NK cells. As shown in Figure 5, addition of BiUII to the cell culture induces the expression of CD95 on CD3–/CD16+NK cells indicating their activation via the Fc-region of BiUII. Consequently, cocultivation of NK cells with allogeneic PCI-1 tumour cells in the presence of BiUII resulted in enhanced tumour-cell killing (Figure 6). We observed that NK cells per se display a remarkable activity against allogeneic cells. However, this cytotoxicity was further enhanced by the addition of BiUII.

BiUII induces the upregulation of costimulatory molecules on dendritic cells

The network of dendritic cells (DCs) is another class of key regulators of immune responses. DCs are potent antigen-presenting cells (Steinman, 1991) and trigger the activation of T cells, e.g. via the CD40-dependent pathway (McLellan et al, 1996). Activation of DCs is characterized by the neoexpression of CD83 (Czerniecki et al, 1997; Zhou and Tedder, 1996) and upregulation of costimulatory molecules (Cella et al, 1996). Thus, DCs are thought to be involved in the generation of cytotoxic T cells (Cella et al, 1996; Ridge et al, 1998).

The objective of this study was to investigate whether DCs are activated by BiUII. DCs were generated from the adherent fraction of PBMCs by incubating these cells for 2 weeks in the presence of IL-4 and GM-CSF. The percentage of DCs in the culture was checked by staining with the DC-specific antibody BMA-X11 and was shown to be > 80% (not shown). The DCs were incubated overnight either with BiUII (100 ng ml^{-1}) or left untreated in cell culture medium only. After 16 h, the expression of surface markers CD83 and CD86 was revealed by FACS analysis. As shown in Figure 7, incubation of DCs in the presence of BiUII leads to the upregulation of both CD83 and the costimulatory signal CD86, indicating the activation of DCs mediated by our bispecific molecule.

DISCUSSION

We demonstrate here that not only the two specific binding arms but also the Fc-region of a bispecific antibody can contribute to activation of immune effector cells and thus to anti-tumour activity. However, binding of Fcγ receptors and activation of FcγR expressing cells strictly depends on the composition of the Fc-region of the bispecific molecule. Mouse IgG2a and rat IgG2b are two evolutionally related potent effector subclasses that, in combination, exert efficient activation of human accessory cells. This is shown by:

- the upregulation of costimulatory molecules and activation markers like CD83, CD86, and CD95
- the upregulation of neopterin synthesis
- the direct phagocytosis of tumour cells by purified monocytes, and
- the direct killing by isolated accessory cells without the contribution of T cells.

Interestingly, PBMCs were only weakly activated by equimolar amounts of the two parental monoclonal antibodies (Zeidler et al, 1999).

Conventional bsAbs are usually composed of one potent subclass like mouse IgG2a or rat IgG2b and a less potent subclass like mouse 1gG1 (de Gast et al, 1995), or even two less potent subclasses (Weiner et al, 1993). As a consequence, the Fc-region of conventional bsAbs is usually not able to activate human accessory cells. Instead, these bispecific molecules bind and activate a single class of effector cell via one of their binding arms. This has the drawback that, in the case of T cells, an isolated activation via the CD3 molecule without appropriate costimulatory signals may cause activation-induced anergy (Daniel et al, 1998). We therefore constructed a bispecific antibody that activates more than one class of immune cell, a situation that much more resembles inflammatory and immune reactions in vivo. We have already shown the potential of such new bsAbs in tumour eradication in an animal

Tumor cell-derived prostaglandin E2 inhibits monocyte function by interfering with CCR5 and Mac-1

REINHARD ZEIDLER,[1] MIKLOS CSANADY,[2] OLIVIER GIRES, STEPHAN LANG, BÄRBEL SCHMITT, AND BARBARA WOLLENBERG

Department of Otolaryngology, University of Munich, 81377 Munich, Germany

ABSTRACT **The cyclooxygenases (COX)-1 and COX-2 are key enzymes in the conversion of arachidonic acid to prostaglandins and other eicosanoids. Whereas COX-1 is expressed ubiquitously, COX-2 is an immediate-early gene often associated with malignant transformation, and a role for the COX enzymes in tumor initiation and promotion is discussed. Nonsteroidal anti-inflammatory drugs (NSAIDs) like aspirin and indomethacin that block COX-1 and -2 have been shown to have beneficial effects for tumor patients. Therefore, these compounds have gained interest also among oncologists. However, the molecular mechanism by which NSAIDs inhibit carcinogenesis is not clearly understood. The prostaglandin-dependent and -independent effect may both account for their antineoplastic action. We show here that tumor cells derived from different tumors regularly produce prostaglandin E_2 (PGE_2) interfering with the function of monocytes. In particular, PGE_2 inhibits the potential of monocytes to migrate in the direction of a chemotactic stimulus and to adhere to endothelial cell. This inhibition is most probably due to a modulation of the chemokine receptor CCR5 and the β2-integrin Mac-1. Both down-regulation of CCR5 and reduced expression of Mac-1 may diminish the potential of peripheral blood monocytes to leave blood vessels and invade target tissues. Since both dysfunctions can be restored with NSAIDs, our findings help to explain the molecular chemopreventive action of NSAIDs on tumor formation and progression.—Zeidler, R., Csanady, M., Gires, O., Lang, S., Schmitt, B., Wollenberg, B. Tumor cell-derived prostaglandin E2 inhibits monocyte function by interfering with CCR5 and Mac-1. *FASEB J.* 14, 661–668 (2000)**

Key Words: immune evasion · prostaglandins · migration · adhesion

THE CYCLOOXYGENASES (COX)-1 and COX-2 are key enzymes of the biosynthetic pathway of prostanoid formation. Both COX-1 and COX-2 are blocked by nonsteroidal anti-inflammatory drugs (NSAIDs) (1). Whereas COX-1 is constitutively expressed in many tissues (2), COX-2 is an immediate-early gene that is induced in inflammatory cells by mitogens, tumor promoters, and cytokines. The cyclooxygenases have become of special interest to oncologists since overexpression of COX-2 was demonstrated in human tumors (3) and epidemiological studies revealed that continuous use of NSAIDs reduces the risk of development of different cancers (4, 5). A causative role for the COX enzymes in tumor development and progression as well as metastatic behavior has also been demonstrated (6–8). Since elevated prostaglandin E2 (PGE_2) levels occur in various cancers (9, 10), inhibition of the COX isoenzymes is probably an important function of NSAIDs (11–13). However, PGE_2-independent effects have also been described (4, 14–16), and the molecular mechanisms of the chemopreventive antineoplastic action of NSAIDs remain unclear. For a summary of COX-dependent and independent NSAIDs actions, we refer to a recently published review (17).

Leukocytes that circulate in the body have to exit the bloodstream in order to exert their immunological function. Leukocytes adhere to endothelial cells at the luminal side of blood vessels, transmigrate, and enter target tissues (18). Adhesion is a complex process that involves a plethora of different molecules like the β2-integrins (CD11a/CD18; CD11b/CD18, and CD11c/CD18) (19). The pivotal immunological significance of β2-integrins is obvious from leukocyte adhesion deficiency type I (LAD-I), a clinical condition caused by a mutation in the β2 common CD18 chain. Patients suffering from LAD-I usually die at a young age due to multiple leukocyte defects (20).

Chemokines are small proteins that function as emergency signals produced locally in response to inflammation and immune responses (21). Chemokines induce chemotaxis of leukocyte subsets and stimulate their adhesion to the endothelium (22).

[1] Correspondence: Department of Otolaryngology, University of Munich, Marchioninistr. 15, 81377 Munich, Germany. E-mail: rzeidler@hno.med.uni-muenchen.de

[2] Present address: Department of Otorhinolaryngolgy, Albert-Szent-Györgyi Medical University, H-6725 Szeged, Hungary.

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Chemokines signal through specific seven-transmembrane domain, G-protein-coupled receptors (23). CCR5, one of these receptors, is expressed on monocytes and certain lymphocytes, and binds macrophage inflammatory protein alpha (MIP1α), MIP1β, and RANTES (24). CCR5 garnered special interest when it was identified as the fusion cofactor for macrophage-tropic HIV (25). The physiological role of CCR5 in activation and migration of monocytes, however, has been studied much less.

Only recently it has been demonstrated that PGE_2 down-regulates CCR5 surface expression on monocytes, rendering these cells resistant to HIV (26). Since the COX isoenzymes are often overexpressed in human tumors and PGE_2 is the major metabolite of arachidonic acid metabolism, we asked whether tumor-derived PGE_2 interferes with the physiological activity and function of monocytes. We show here that PGE_2 derived from human carcinoma cell lines causes down-regulation of the surface expression of CCR5 and the adhesion molecule Mac-1 on monocytes. Down-regulation results in a reduced competence of monocytes to respond to a chemoattractant (MIP-1β) and to adhere to endothelial cells. In addition, conditioned tumor supernatants induce the high level production of interleukin 10 (IL-10) and tumor necrosis factor α (TNF-α) in monocytes. These effects can be inhibited by the NSAIDs aspirin and indomethacin. Since adhesion and migration are also pivotal steps in the recruitment of effector cells into the tumor stroma and the subsequent eradication of tumor cells (27), their inhibition may have implications on tumor development. Our findings provide a new molecular explanation for the beneficial effects of aspirin and indomethacin on tumor incidence and may have clinical consequences for the treatment of cancer patients.

MATERIALS AND METHODS

Cell lines, cytokines, and PGE2

FaDu (HTB-43; ATCC, Manassas, Va.) and PCI-1 (a gift of Dr. T. Whiteside, Pittsburgh, Pa.) are cell lines derived from squamous cell carcinoma of the head and neck, and GHD-1 is a permanent cell line derived from a hypopharynx carcinoma that was established in our laboratory. MCF-7 (HTB-22; ATCC) is a human mammary carcinoma and HCT-8 (CCL-244; ATCC) is a human colon carcinoma cell line. All cell lines used were maintained as continuously growing monolayers in DMEM with 10% fetal calf serum (FCS) (both from Seromed, Berlin, Germany). Recombinant human TNF-α and IL-10 were from Boehringer Mannheim (Mannheim, Germany); PGE_2 was from Sigma (Munich, Germany).

Generation of cell-free tumor cell supernatants

Tumor cells were seeded at 10^5 cells/ml and grown for 2 days in DMEM at 37°C. Supernatants were harvested, centrifuged, and passed through a 0.2 μm Acrodisc low protein binding filter (Gelman Sciences, Ann Arbor, Mich.). IL-10, TNF-α, and PGE_2 production was determined with commercial ELISA assay (R&D Systems; Wiesbaden, Germany) according to the manufacturer's instructions. PGE_2 production given in **Table 1** represents the mean of three different experiments (SD <20%).

TABLE 1. *PGE_2 production in various tumor cell supernatants and their effects on CCR5 and Mac-1 expression on monocytes as observed by FACS*[a]

Cell line	PGE_2 (pg/ml)	CCR5	Mac-1
FaDu	200	↓	↓
PCI-1	>4.000	↓	↑
GHD-1	0	—	—
HCT-8	185	↓	↓
MCF-7	165	↓	↓

[a] All tumor cells tested produce PGE_2, albeit at varying concentrations. Given are the mean values of three independent experiments. Samples were used as duplicates, SD was below 20%. The influence of these conditioned supernatants on CCR5 and Mac-1 expression on primary monocytes is heterogeneous and argues for the presence of additional immunomodulating factors other than PGE_2. For determination of CCR5 and Mac-1 expression, primary monocytes derived from healthy volunteers were cultivated for 2 days in conditioned media prior to FACS analysis. The up-regulation of Mac-1 after incubation of monocytes in PCI-1 supernatants is not understood.

FACS analysis

For FACS analysis, 10^5 cells were incubated with the primary antibody for 30 min on ice in phosphate-buffered saline (PBS)/5% FCS. The cells were washed twice in PBS and incubated for another 30 min with the second FITC-labeled antibody. After two final washings, propidiumiodide was added and flow cytometry was performed using a FACSCalibur cytometer and the CellQuest analysis program (Becton Dickinson, Heidelberg, Germany).

Adhesion assay

For monocyte adhesion, peripheral blood monocytes were incubated for 2 days in either DMEM or 100% conditioned tumor cell supernatants. Primary endothelial cells from umbilical blood cords (10,000 cells/per well) were seeded in 96-well plates at half-confluency and kept for 2 days in endothelial cell growth medium (Promocell, Heidelberg, Germany). After 2 days, endothelial cells had reached confluency. Monocytes were labeled for 30 min at 37°C with 20 μM of the fluorochromic dye CMFDA (Molecular Probes, Eugene, Oreg.) and washed twice; 2×10^4 monocytes were than added to the endothelial cell layer for 30 min to promote adherence. Plates were washed three times and supernatant was almost completely removed. The 96-well plate was then covered, inverted, and centrifuged for 10 min at 500 *g* at room temperature. The number of adhering monocytes was determined by measuring fluorescence at 525 nm in a Wallac 1420 Victor multilabel counter (Wallac, Turku; Finland). For adhesion to recombinant human intercellular adhesion molecule 1 (ICAM-1), 293 cells were transfected with an expression plasmid encoding a fusion protein of the Fc part of a human immunoglobulin (IgG1) and ICAM-1 (Fc/ICAM-1; a generous gift of Dr. W. Kolanus, Munich) and supernatants were collected 4 days after trans-

fection. Culture dishes (Falcon 1008) were first coated for 1.5 h with a human IgG-specific antibody (5 μg/ml; Dianova, Hamburg; Germany) in 50 mM Tris-Cl, pH 9.4 and then for 4 h at room temperature with the supernatant from transfected 293 cells. Dishes were washed twice to remove unbound ICAM-1 and monocytes were added for 2 h. After two final washings, adherent cells were trypsinized and counted.

Monocyte migration

For cell migration assays, 2×10^6 monocytes were precultivated for 1 day in either DMEM or 100% conditioned FaDu-SN. Monocytes were then placed on 8 μm pore size polycarbonate filters (Nunc, Roskilde, Denmark) and allowed to transmigrate for 4 h in the direction of MIP1β (20 ng/ml; R&D Systems, Heidelberg, Germany) in the lower chamber. Migrated cells were pelleted, stained with Giemsa black, and counted under light microscopy. Mean values of migrated cells were calculated from 3 wells/supernatant.

RESULTS

Tumor cell supernatants induce the production of IL-10 and TNF-α

Tumor samples derived from head and neck cancer and other locations, as well as permanent tumor cell lines, have been shown to produce PGE_2 (28–30). Most of the tumor cell lines we used for our investigations also produce PGE_2 (Table 1). Since PGE_2 induces IL-10 production (30), we first asked whether these tumor cell lines also synthesize this immunosuppressive cytokine. ELISA assays performed with conditioned tumor cell supernatants (Tu-SN), however, revealed that none of these cancer cell lines produce detectable amounts of IL-10 (**Fig. 1**). The fact that IL-10 synthesis has been described in freshly excised tumors (31–33) prompted us to investigate whether these tumor cells induce IL-10 production in primary peripheral blood monocytes. Therefore, cultivated monocytes were kept in conditioned tumor supernatants or DMEM cell culture medium for 2 days. Monocytes *per se* produce only small amounts of IL-10 but synthesis of this cytokine was dramatically induced by cultivation of monocytes in tumor supernatants for 2 days (Fig. 1).

TNF-α is a cytokine that has been described to be induced in monocytes stimulated with human cancer cells (34). TNF-α mostly displays proinflammatory properties, but is also able to block T cell proliferation (28, 35). We again performed ELISA assays that demonstrated that conditioned Tu-SN not only induce IL-10 but also induce the production of TNF-α in monocytes (Fig. 1).

Tumor supernatants down-regulate surface expression of CCR5

CCR5 is a chemokine receptor expressed on monocytes and certain T lymphocytes (24, 36). It has been

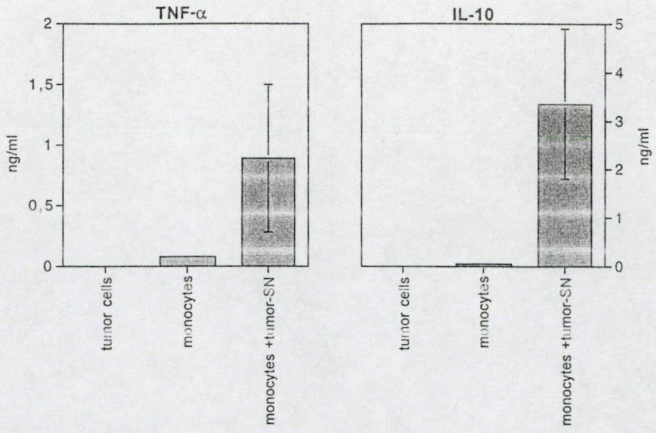

Figure 1. Supernatants of tumor cell lines derived from head and neck, breast, and colon cancer induce IL-10 and TNF-α production by primary monocytes. All tumor cell lines tested neither produced IL-10 nor TNF-α *per se*, and only small amounts were detectable in primary monocytes. However, identical conditioned tumor SN induced the production of both cytokines in monocytes after cultivation in 100% conditioned media for 2 days. Mean values of three experiments are shown; samples were used in duplicates. The GHD-1 cancer cell line that does not produce PGE_2 (Table 1) does not induce cytokine production (not shown).

identified as a coreceptor for HIV entry (25), but its physiological role is activation and regulation of responses to chemokines. To investigate the influence of tumor cell SN on the surface expression of CCR5, we cultivated freshly isolated monocytes for 2 days in different conditioned tumor cell media and investigated CCR5 by FACS analysis. Vitality of monocytes was usually >90% and was not influenced by Tu-SN as tested by trypan blue exclusion. As demonstrated in **Fig. 2*A***, the incubation with Tu-SN led to a significant down-regulation of CCR5 in comparison to incubation with cell culture medium. Supernatants from a tumor cell line that does not produce PGE_2 (GHD-1) did not show this effect (data not shown). Most likely, this effect is mediated by PGE_2 present in the tumor cell supernatants, since the observed effect can be inhibited by aspirin (Fig. 2*B*) and indomethacin (not shown) and simulated with purified PGE_2 (Fig. 2*C*).

Tu-SN reduce the chemotaxis of monocytes

Chemokines are produced locally in response to infections and immune reactions. The migration of immune cells in the direction of higher chemokine concentrations is necessary for efficient immune responses (21, 37, 38). Since MIP-1β, which is produced by activated T cells (21), binds to CCR5, and Tu-SN down-regulate CCR5, we performed a chemotaxis assay in order to determine whether CCR5 down-regulation results in a reduced ability of primary monocytes to migrate in the direction of higher MIP-1β concentrations. Therefore, primary mono-

cytes were cultivated for 1 day in either FaDu-SN or cell culture medium. Migration was then performed against MIP-1β (20 ng/ml) for 3 h through 8 μm pore filters. As pointed out in **Fig. 3**, the pretreatment of monocytes with FaDu-SN significantly ($P<0.003$) reduces the number of migrated monocytes to ~50% compared with DMEM. Migration inhibition was partially abrogated by cultivation of FaDu cells in the presence of 1 mM aspirin.

Tu-SN down-regulate adhesion molecules on monocytes and inhibit adhesion

As demonstrated in a recent study, the number of immune cells in the tumor infiltrate is increased after administration of indomethacin (39). Since adhesion to the endothelium is another pivotal step for leukocytes in order to leave the bloodstream, we investigated whether Tu-SN also inhibit the function of the β2-integrin Mac-1 on monocytes. We incubated monocytes for 2 days in either cell culture medium or conditioned tumor supernatants and investigated surface expression of Mac-1 by FACS. We found that both chains of Mac-1, CD11b and CD18, were clearly down-regulated after incubation in FaDu-SN (**Fig. 4**). This modulation was not observed when FaDu-SN were generated in the presence of aspirin or indomethacin (not shown).

The main ligand for Mac-1 is ICAM-1 present on endothelial cells, and this interaction is pivotal for adhesion and extravasation of monocytes (40). Therefore, we next investigated whether down-regulation of Mac-1 has consequences for the adhesion of monocytes to recombinant ICAM-1. To this end, cell culture dishes were coated with the fusion protein Fc/ICAM-1 and monocytes preincubated with FaDu-

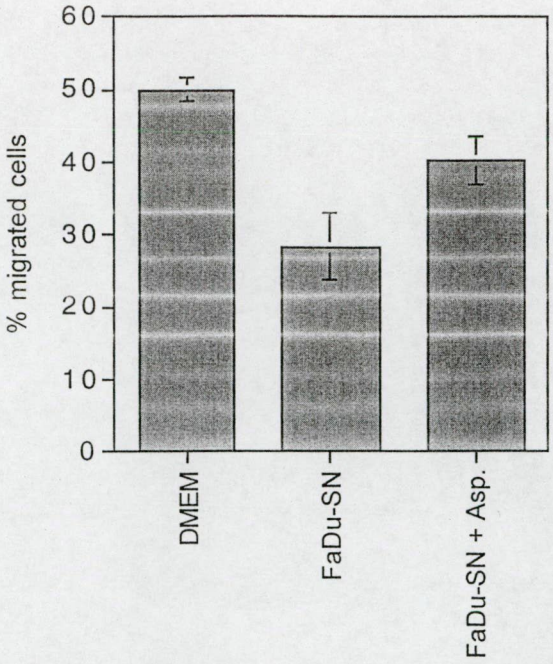

Figure 3. FaDu-derived factors interfere with the migration of monocytes. Isolated monocytes were cultivated for 1 day in either conditioned FaDu-SN or cell culture medium (DMEM). Transmigration of monocytes through 8 μm pore size filters was then performed against MIP-1β (20 ng/ml) for 4 h. Cells that reached the lower chamber were stained with Giemsa black and counted under light microscopy. Migration was reduced to almost 50% after incubation in FaDu-SN and was much less inhibited, when FaDu-SN were generated in the presence of 1 mM aspirin (FaDu-SN + Asp). Results represent the mean of 4 values. $P < 0:003$ (paired Student's test). A representative result of three independent experiments is shown.

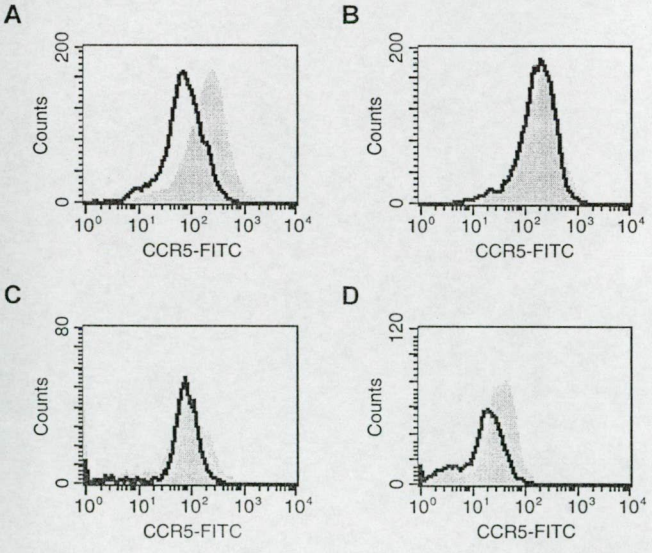

Figure 2. Tumor-SN down-regulate the expression of CCR5 on monocytes. *A)* Incubation of monocytes for 2 days with FaDu-SN resulted in a clear down-regulation of CCR5 (black line) in contrast to cell culture medium (gray trace). This effect could be abolished, when FaDu cells had been grown in the presence of 1 mM aspirin (*B*) or 13 μM indomethacin (*C*). CCR5 was similarly down-regulated when purified 10^{-5} M PGE_2 was added to cell culture medium (*D*).

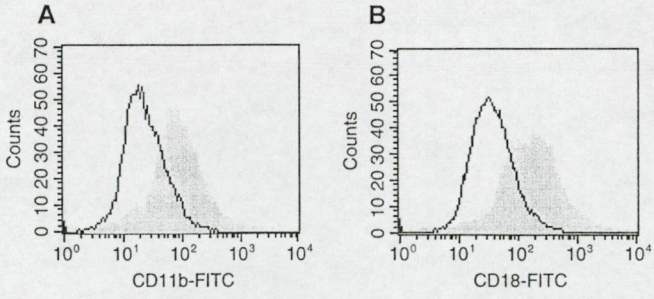

Figure 4. Down-regulation of Mac-1 expression after incubation of monocytes in FaDu-SN. Monocytes were incubated for 2 days with either FaDu-SN (black line) or cell culture medium (gray trace) and investigated for Mac-1 expression. Both chains of the molecule, CD11b and CD18, were clearly down-regulated after incubation in FaDu-SN in comparison to cell culture medium. Similar results were observed after addition of purified PGE2 to the medium. FaDu-SN generated in the presence of aspirin (1 mM) and Indomethacin (13 μM) did not show this effect (data not shown).

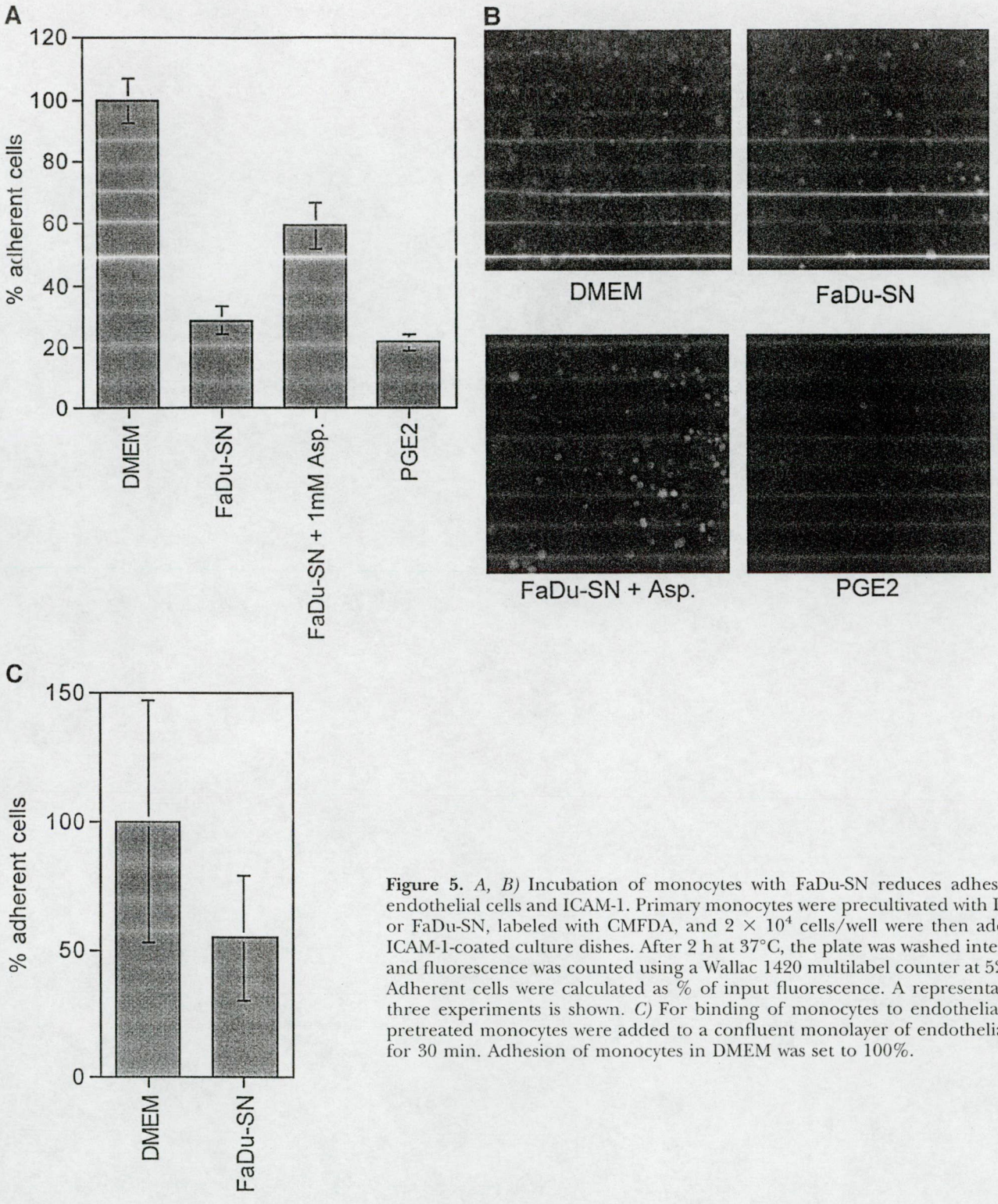

Figure 5. *A, B)* Incubation of monocytes with FaDu-SN reduces adhesion to endothelial cells and ICAM-1. Primary monocytes were precultivated with DMEM or FaDu-SN, labeled with CMFDA, and 2×10^4 cells/well were then added to ICAM-1-coated culture dishes. After 2 h at 37°C, the plate was washed intensively and fluorescence was counted using a Wallac 1420 multilabel counter at 525 nm. Adherent cells were calculated as % of input fluorescence. A representative of three experiments is shown. *C)* For binding of monocytes to endothelial cells, pretreated monocytes were added to a confluent monolayer of endothelial cells for 30 min. Adhesion of monocytes in DMEM was set to 100%.

SN, which had been generated in the presence of 1 mM aspirin, purified PGE_2, or cell culture medium medium only. It became clear that FaDu-SN and purified PGE_2 strongly inhibited binding of monocytes to ICAM-1, whereas FaDu-SN that were generated in the presence of aspirin had a much less dramatic effect (**Fig. 5*A, B***).

In an additional set of experiments, we investigated whether tumor supernatants also interfere with the adhesion of monocytes to endothelial cells. We isolated human umbilical cord endothelial cells and monocytes and pretreated them with DMEM or FaDu-SN. Monocytes were then labeled with CMFDA and put onto the endothelial cell layer for 30 min. After intensive washings, adherent monocytes were counted with a fluorometer. As shown for recombinant ICAM-1, FaDu-SN also reduced the adhesion of monocytes to endothelial cells (Fig. 5*C*).

7. Tsujii, M., Kawano, S., and DuBois, R. N. (1997) Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. *Proc. Natl. Acad. Sci. USA* **94**, 3336–3340
8. Murata, H., Kawano, S., Tsuji, S., Tsuji, M., Sawaoka, H., Kimura, Y., Shiozaki, H., and Hori, M. (1999) Cyclooxygenase-2 overexpression enhances lymphatic invasion and metastasis in human gastric carcinoma. *Am. J. Gastroenterol.* **94**, 451–455
9. McLemore, T. L., Hubbard, W. C., Litterst, C. L., Liu, M. C., Miller, S., McMahon, N. A., Eggleston, J. C., and Boyd, M. R. (1988) Profiles of prostaglandin biosynthesis in normal lung and tumor tissue from lung cancer patients. *Cancer Res.* **48**, 3140–3147
10. Rigas, B., Goldman, I. S., and Levine, L. (1993) Altered eicosanoid levels in human colon cancer. *J. Lab. Clin. Med.* **122**, 518–523
11. Oshima, M., Dinchuk, J. E., Kargman, S. L., Oshima, H., Hancock, B., Kwong, E., Trzaskos, J. M., Evans, J. F., and Taketo, M. M. (1996) Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). *Cell* **87**, 803–809
12. Okajima, E., Denda, A., Ozono, S., Takahama, M., Akai, H., Sasaki, Y., Kitayama, W., Wakabayashi, K., and Konishi, Y. (1998) Chemopreventive effects of nimesulide, a selective cyclooxygenase-2 inhibitor, on the development of rat urinary bladder carcinomas initiated by N-butyl-N-(4-hydroxybutyl)nitrosamine. *Cancer Res.* **58**, 3028–3031
13. Rao, C. V., Rivenson, A., Simi, B., Zang, E., Kelloff, G., Steele, V., and Reddy, B. S. (1995) Chemoprevention of colon carcinogenesis by sulindac, a nonsteroidal anti-inflammatory agent. *Cancer Res.* **55**, 1464–1472
14. Kopp, E., and Ghosh, S. (1994) Inhibition of NF-kappa B by sodium salicylate and aspirin. *Science* **265**, 956–959
15. Dong, Z., Huang, C., Brown, R. E., and Ma, W. Y. (1997) Inhibition of activator protein 1 activity and neoplastic transformation by aspirin. *J. Biol. Chem.* **272**, 9962–9970
16. Piazza, G. A., Rahm, A. K., Finn, T. S., Fryer, B. H., Li, B. H., Stoumen, A. L., Pamukcu, R., and Ahnen, D. J. (1997) Apoptosis primarily accounts for the growth-inhibitory properties of sulindac metabolites and involves a mechanism that is independent of cyclooxygenase inhibition, cell cycle arrest, and p53 induction. *Cancer Res.* **57**, 2452–2459
17. Shiff, S. J., and Rigas, B. (1999) The role of cyclooxygenase inhibition in the antineoplastic effects of nonsteroidal antiinflammatoy drugs (NSAIDs). *J. Exp. Med.* **190**, 445–450
18. Springer, T. A. (1995) Traffic signals on endothelium for lymphocyte recirculation and leukocyte emigration. *Annu. Rev. Physiol.* **57**, 827–872
19. Carlos, T. M., and Harlan, J. M. (1994) Leukocyte–endothelial adhesion molecules. *Blood* **84**, 2068–2101
20. Gahmberg, C. G., Tolvanen, M., and Kotovuori, P. (1997) Leukocyte adhesion—structure and function of human leukocyte beta2-integrins and their cellular ligands. *Eur. J. Biochem.* **245**, 215–232
21. Rollins, B. J. (1997) Chemokines. *Blood* **90**, 909–928
22. Vaddi, K., and Newton, R. C. (1994) Regulation of monocyte integrin expression by beta-family chemokines. *J. Immunol.* **153**, 4721–4732
23. Murphy, P. M. (1994) The molecular biology of leukocyte chemoattractant receptors. *Annu. Rev. Immunol.* **12**, 593–633
24. Raport, C. J., Gosling, J., Schweickart, V. L., Gray, P. W., and Charo, I. F. (1996) Molecular cloning and functional characterization of a novel human CC chemokine receptor (CCR5) for RANTES, MIP-1beta, and MIP-1alpha. *J. Biol. Chem.* **271**, 17161–17166
25. Choe, H., Farzan, M., Sun, Y., Sullivan, N., Rollins, B., Ponath, P. D., Wu, L., Mackay, C. R., LaRosa, G., Newman, W., Gerard, N., Gerard, C., and Sodroski, J. (1996) The beta-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell* **85**, 1135–1148
26. Thivierge, M., Le Gouill, C., Tremblay, M. J., Stankova, J., and Rola Pleszczynski, M. (1998) Prostaglandin E2 induces resistance to human immunodeficiency virus-1 infection in monocyte-derived macrophages: downregulation of CCR5 expression by cyclic adenosine monophosphate. *Blood* **92**, 40–45
27. Morisaki, T., and Torisu, M. (1991) Enhanced adherence activity of OK-432-induced peritoneal neutrophils to tumor cells correlates to their increased expression of CD11b/CD18. *Clin. Immunol. Immunopathol.* **59**, 474–486
28. Alleva, D. G., Burger, C. J., and Elgert, K. D. (1994) Tumor-induced regulation of suppressor macrophage nitric oxide and TNF-alpha production. Role of tumor-derived IL-10, TGF-beta, and prostaglandin E2. *J. Immunol.* **153**, 1674–1686
29. Snyderman, C. H., Klapan, I., Milanovich, M., Heo, D. S., Wagner, R., Schwartz, D., Johnson, J. T., and Whiteside, T. L. (1994) Comparison of in vivo and in vitro prostaglandin E2 production by squamous cell carcinoma of the head and neck. *Otolaryngol.-Head Neck Surg.* **111**, 189–196
30. Huang, M., Sharma, S., Mao, J. T., and Dubinett, S. M. (1996) Non-small cell lung cancer-derived soluble mediators and prostaglandin E2 enhance peripheral blood lymphocyte IL-10 transcription and protein production. *J. Immunol.* **157**, 5512–5520
31. Pisa, P., Halapi, E., Pisa, E. K., Gerdin, E., Hising, C., Bucht, A., Gerdin, B., and Kiessling, R. (1992) Selective expression of interleukin 10, interferon gamma, and granulocyte-macrophage colony-stimulating factor in ovarian cancer biopsies. *Proc. Natl. Acad. Sci. USA* **89**, 7708–7712
32. Yamamura, M., Modlin, R. L., Ohmen, J. D., and Moy, R. L. (1993) Local expression of antiinflammatory cytokines in cancer. *J. Clin. Invest.* **91**, 1005–1010
33. Nakagomi, H., Pisa, P., Pisa, E. K., Yamamoto, Y., Halapi, E., Backlin, K., Juhlin, C., and Kiessling, R. (1995) Lack of interleukin-2 (IL-2) expression and selective expression of IL-10 mRNA in human renal cell carcinoma. *Int. J. Cancer* **63**, 366–371
34. Zembala, M., Siedlar, M., Ruggiero, I., Wieckiewicz, J., Mytar, B., Mattei, M., and Colizzi, V. (1994) The MHC class-II and CD44 molecules are involved in the induction of tumour necrosis factor (TNF) gene expression by human monocytes stimulated with tumour cells. *Int. J. Cancer* **56**, 269–274
35. Van Bergen, C. A. M., Smit, W. M., Van Sluijters, D. A., Rijnbeek, M., Willemze, R., and Falkenburg, J. H. F. (1996) Interleukin-10, interleukin-12, and tumor necrosis factor-alpha differentially influence the proliferation of human CD8(+) and CD4(+) T-cell clones. *Ann. Hematol.* **72**, 245–252
36. Loetscher, P., Uguccioni, M., Bordoli, L., Baggiolini, M., Moser, B., Chizzolini, C., and Dayer, J. M. (1998) CCR5 is characteristic of Th1 lymphocytes. *Nature (London)* **391**, 344–345
37. Taub, D. D., Sayers, T. J., Carter, C. R. and Ortaldo, J. R. (1995) Alpha and beta chemokines induce NK cell migration and enhance NK-mediated cytolysis. *J. Immunol.* **155**, 3877–3888
38. Xu, L. L., Warren, M. K., Rose, W. L., Gong, W., and Wang, J. M. (1996) Human recombinant monocyte chemotactic protein and other C-C chemokines bind and induce directional migration of dendritic cells in vitro. *J. Leukoc. Biol.* **60**, 365–371
39. Cross, D. S., Platt, J. L., Juhn, S. K., Bach, F. H., and Adams, G. L. (1992) Administration of a prostaglandin synthetase inhibitor associated with an increased immune cell infiltrate in squamous cell carcinoma of the head and neck. *Arch. Otolaryngol. Head Neck Surg.* **118**, 526–528
40. Arnaout, M. A. (1990) Structure and function of the leukocyte adhesion molecule CD11/CD18. *Blood* **75**, 1037–1050
41. Goodwin, J. S., and Ceuppens, J. (1983) Regulation of the immune response by prostaglandins. *J. Clin. Immunol.* **3**, 295–315
42. Kune, G. A., Kune, S., and Watson, L. F. (1988) Colorectal cancer risk, chronic illnesses, operations, and medications: case control results from the Melbourne Colorectal Cancer Study. *Cancer Res.* **48**, 4399–4404
43. Schreinemachers, D. M., and Everson, R. B. (1994) Aspirin use and lung, colon, and breast cancer incidence in a prospective study. *Epidemiology* **5**, 138–146
44. Rollins, B. J., and Sunday, M. E. (1991) Suppression of tumor formation in vivo by expression of the JE gene in malignant cells. *Mol. Cell Biol.* **11**, 3125–3131
45. Luster, A. D., and Leder, P. (1993) IP-10, a -C-X-C- chemokine, elicits a potent thymus-dependent antitumor response in vivo. *J. Exp. Med.* **178**, 1057–1065
46. Huang, S., Singh, R. K., Xie, K., Gutman, M., Berry, K. K., Bucana, C. D., Fidler, I. J., and Bar Eli, M. (1994) Expression of the JE/MCP-1 gene suppresses metastatic potential in murine colon carcinoma cells. *Cancer Immunol. Immunother.* **39**, 231–238
47. Dilloo, D., Bacon, K., Holden, W., Zhong, W., Burdach, S., Zlotnik, A., and Brenner, M. (1996) Combined chemokine and

cytokine gene transfer enhances antitumor immunity. *Nat. Med.* **2**, 1090–1095
48. Fioretti, F., Fradelizi, D., Stoppacciaro, A., Ramponi, S., Ruco, L., Minty, A., Sozzani, S., Garlanda, C., Vecchi, A., and Mantovani, A. (1998) Reduced tumorigenicity and augmented leukocyte infiltration after monocyte chemotactic protein-3 (MCP-3) gene transfer: perivascular accumulation of dendritic cells in peritumoral tissue and neutrophil recruitment within the tumor. *J. Immunol.* **161**, 342–346
49. Zhang, L., T. Yoshimura, and Graves, D. T. (1997) Antibody to Mac-1 or monocyte chemoattractant protein-1 inhibits monocyte recruitment and promotes tumor growth. *J. Immunol.* **158**, 4855–4861
50. Punnonen, J., De Waal Malefyt, R., Van Vlasselaer, P., Gauchat, J. F., and De Vries, J. E. (1993) IL-10 and viral IL-10 prevent IL-4-induced IgE synthesis by inhibiting the accessory cell function of monocytes. *J. Immunol.* **151**, 1280–1289
51. Taga, K., Mostowski, H., and Tosato, G. (1993) Human interleukin-10 can directly inhibit T-cell growth. *Blood* **81**, 2964–2971
52. Zeidler, R., Eissner, G., Meissner, P., Uebel, S., Tampé, R., Lazis, S., and Hammerschmidt, W. (1997) Downregulation of tap1 in B-lymphocytes by cellular and Epstein-Barr virus encoded IL-10. *Blood* **90**, 2390–2397
53. Sica, A., Saccani, A., Borsatti, A., Power, C. A., Wells, T. N., Luini, W., Polentarutti, N., Sozzani, S., and Mantovani, A. (1997) Bacterial lipopolysaccharide rapidly inhibits expression of C-C chemokine receptors in human monocytes. *J. Exp. Med.* **185**, 969–974
54. Young, M. R., Wright, M. A., Lozano, Y., Matthews, J. P., Benefield, J., and Prechel, M. M. (1996) Mechanisms of immune suppression in patients with head and neck cancer: influence on the immune infiltrate of the cancer. *Int. J. Cancer* **67**, 333–338

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