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Comparison of retroviral p15E-related factors and interferon α in head and neck cancer

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Abstract. Head and neck squamous cell carcinomas (HNscc) produce low-molecular-mass factors (low-M_r factors, $M_r \leq 25{,}000$), which are antigenically related to the immunosuppressive retroviral transmembrane envelope protein p15E. These P15E-related tumour factors are thought to be responsible for some immunological impairments found in these patients (particularly the defective monocyte chemotaxis). A sequential and functional homology has been reported to exist between a bioactive fragment of interferon α (IFNα) and the putative immunosuppressive region of retroviral p15E (CKS-17). In this study we investigated (a) a possible functional and structural relationship between p15E and IFNα, and (b) the presence of and the relationship between p15E-related low-M_r factors and IFNα in HNscc patients. We report the following results. (a) Recombinant human (rhu) IFNα was able to inhibit monocyte chemotaxis. (b) The anti-p15E antibodies crossreacted with rhuIFNα in a dot-blot technique; however, the anti-IFNα antibodies did not crossreact with disrupted murine leukaemia virus (p15E source). (c) Low-M_r factors ($n = 8-11$) prepared from the sera of HNscc patients, which inhibit the monocyte chemotactic responsiveness, could be adsorbed by the anti-p15E antibodies as well as by the anti-IFNα antibodies. However, the abilities of the factors to adsorb to the two categories of antibodies (namely, anti-p15E and anti-IFNα) did not correlate. (d) Immunohistochemically we found IFNα-related epitopes, in almost all HNscc specimens studied (17/18), in locations distinctive from those of p15E-related factors. The anti-IFNα antibodies used in this study mainly reacted with basal epithelial cells close to the basal membrane, the prickle and granular cells of the squamous cell carcinomas. The anti-p15E antibodies mainly reacted with corneal layers, the granular and prickle cells, and did not react with basal epithelial cells. Our findings suggest that the immunosuppressive factors produced by HNscc cells are heterogeneous and p15E- and/or IFNα-related.

Key words: p15E – IFNα – Monocyte – Immunosuppression – Head and neck cancer

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Introduction

Head and neck squamous cell carcinoma (HNscc) patients show several defects in cell-mediated immunity [1], most notably in chemotactic responsiveness of blood monocytes [2]. Removal of the HNscc restores this defective chemotactic responsiveness [3], indicating that the tumor produces immunosuppressive factors. Indeed, low-molecular-mass factors (low-M_r factors, $M_r \leq 25{,}000$), which inhibit monocyte and macrophage responses to chemotaxins, have been detected in tumor specimens [4, 5] and sera [6–8] of HNscc patients. Interestingly, the effect of these factors has been shown to be abrogated with monoclonal antibodies (mAb) directed against a retroviral immunosuppressive protein, p15E. This was taken as evidence that these low-M_r factors were structurally related to p15E, and possibly involved in hampering the defense immune response towards cancer cells.

P15E is the transmembrane envelope protein of several animal leukaemogenic retroviruses, such as murine leukaemia virus (MuLV) and feline leukaemia virus (FeLV), and is well known for its immunosuppressive actions (reviewed in [9, 10]). A synthetic 17-amino-acid peptide (CKS-17), homologous to a highly conserved region in several retroviral transmembrane envelope proteins [11], has similar immunosuppressive actions, amongst which are inhibition of monocyte chemotactic responsiveness [12], interleukin-1(IL-1)-mediated monocyte tumour killing [13], the respiratory burst of human monocytes [14], the mitogen- and alloantigen-stimulated proliferation of human

lymphocytes [15, 16], immunoglobulin synthesis by B lymphocytes [17], interferon γ (IFNγ) production [18], human natural killer (NK) cell activity [19], and in vivo delayed-type hypersensitivity (DTH) to sheep red blood cells in mice [20].

Recently, Ruegg et al. [21] identified a CKS-17-like conserved region in human interferon α (IFNα), a small fragment which, when presented as a synthetic peptide, inhibited proliferation of both the Daudi lymphoblastoid cell line and anti-CD3-stimulated human T lymphocytes. This fragment exhibited a high degree of amino acid sequential identity (70%) to CKS-17. Recombinant human (rhu) IFNα (subtype 2) has a similar antiproliferative action. Wegemer et al. [22] have found a peptide (P+:ISP) comprised of CKS-17 linked to another p15E-derived sequence (rich in polar positive residues) from FeLV, which like IFNα showed antiviral activity on feline- and murine-virus-infected cell lines. These authors postulated that some of the biological effects of P+:ISP are mediated through a common pathway shared with IFNα.

Since an immunosuppressive CKS-17-like sequence is also present in IFNα, we tried to establish a homologous (chemotactic inhibitory) action between p15E and IFNα (M_r = 19,000–26,000 [23]). To verify this possible homologous action, we investigated the effect of rhuIFNα on the chemotactic responsiveness of healthy donor monocytes (measured with the monocyte polarization assay, which represents an early event of the chemotactic responsiveness [3, 24]). We also established the reactivity of the anti-p15E/CKS-17 antibodies against rhuIFNα using a dot-blot technique, and vice versa (anti-IFNα antibodies to retroviral p15E). Furthermore, we were interested in whether the p15E-related HNscc low-M_r factors showed IFNα relatedness. We therefore analysed the immunoreactivity of anti-IFNα antibodies on tumour specimens and sera of HNscc patients, and compared these data with the immunoreactivity of the anti-p15E/CKS-17 antibodies.

Materials and methods

Patients. We studied 18 male and 7 female patients, aged 37–72 years, with HNscc of the oral cavity, larynx and oro- and hypopharynx; stages T1N2–T4N2. All patients were treated by surgery and/or radiotherapy at the department of ear, nose and throat/head and neck surgery of the Dijkzigt University Hospital, Rotterdam, the Netherlands.

After surgery, neoplastic tissues were snap-frozen in liquid nitrogen and stored at –70° C. Serum samples were obtained by venapuncture and stored at –20° C until use.

Monoclonal and polyclonal antibodies. Anti-(MuLV)p15E mAb used were 19F8 [25, 26] (IgG2b) and 4F5 [26, 27] (IgG2a). Anti-CKS-17 mAb, directed against CKS-17-cysteine coupled to keyhole limpet haemocyanin (KLH), have been developed at our department (Dr. M. S. Lang, manuscript in preparation). Two anti-CKS-17 mAb were used, ER-IS5 (IgG2b) and ER-IS1 (IgM; called CK1 in [26]).

Anti-rhuIFNα2b mAb (IgG1) and polyclonal antibodies (pAb) (IgGs) were a generous gift from Dr. P. van der Meyde, TNO Health Research, Rijswijk, the Netherlands.

Control antibodies used were: IgG2a and IgG1 (isotype controls: Becton Dickinson), IgG2b (ICN 64-337 (myeloma source), ICN Biomedical Inc., Costa Mesa, Calif., USA), IgM (blood group antigen A, Dakopatts, Copenhagen, Denmark) and IgGs (rabbit anti-ox red blood cells, Seralab Ltd., Crawley Down, GB).

Table 1. Antibodies and their protein concentrations used in different methods

Antibodies	Final protein concentrations (μg/ml)		
	A	B	C
4F5	25	25	25
19F8	25	25	25
4F5/19F8	ND	25/25	ND
ER-IS5	25	25	25
ER-IS1	25	ND	25
Anti-IFNα (mAb)	30	30	15
Anti-IFNα (pAb)	10	10	5
Control IgG2a	25	25	25
Control IgG2b	25	25	25
Control IgG1	30	30	15
Control IgM	25	ND	25
Control IgGs	10	10	5
RaM-PO	10	ND	10
SaR-PO	10	ND	10

A, Immunohistochemistry; B, adsorption experiments; C, dot-blot technique; ND, not determined; RaM-PO and SaR-PO, peroxidase-labelled rabbit anti-(mouse Ig) and swine anti-(rabbit Ig)

Secondary antibodies used were swine anti-(rabbit Ig) and rabbit anti-(mouse Ig) conjugated to horseradish peroxidase (SaR-PO and RaM-PO) (Dakopatts, Copenhagen, Denmark).

Immunohistochemistry. Sections of 4 μm thickness were cut using a cryostat, mounted on glass slides, air-dried and acetone-fixed for 10 min. These sections were pre-incubated with tenfold-diluted normal sera (Dakopatts, Copenhagen, Denmark) depending on the primary antibody step used; normal rabbit serum (using mAb) and normal swine serum (using pAb). The sections were subsequently incubated for 60 min with the different specific antibodies mentioned above (and controls), washed three times with phosphate-buffered saline (PBS, pH 7.4), and incubated for 30 min with peroxidase-conjugated immunoglobulins. Subsequently the sections were stained using chromagen 1,2-diaminobenzidine (Sigma Chemical Corp., St. Louis, USA), and weakly counterstained with haematoxylin. All the steps described were performed at 21° C, and antibody dilutions were made in PBS/1% bovine serum albumin (Sigma Chemical Co., St. Louis, USA).

Antibody protein concentrations were used as shown in Table 1.

Isolation of peripheral blood monocytes. Monocytes from healthy donors were isolated via counterflow elutriation centrifugation [7, 12]. These almost pure monocyte fractions were used to test the effects of low-M_r factors in sera of HNscc patients and rhuIFNα2b on the polarization assay (see below). In brief, mononuclear cells were separated from 450 ml whole blood of healthy individuals via Percoll centrifugation (20 min, 1000 *g*). Thereafter, the mononuclear cells were injected into an elutriation centrifugation system (Beckman J21 centrifuge with a JE-6 elutriation rotor). The elutriation medium was PBS with 13 mM trisodium citrate and 0.5% human albumin. To separate the different cell populations, the flow rate was kept constant at 20 ml/min, while the rotor speed decreased from 4000 to 0 rpm. The fraction collected at 2500 rpm contained 93%–97% non-specific-esterase-positive cells [28]. This fraction was used in further experiments after storage in liquid nitrogen as described previously [12].

The monocyte polarization assay. The Cianciolo and Snyderman [24] assay for monocyte polarization was performed with slight modifications [3]. The outcomes of the assay correlate well with the outcomes of the conventional Boyden chamber assay to measure chemotaxis to formylmethionylleucylphenylalanine (fMetLeuPhe) and casein [3, 24]. Aliquots (0.2 ml) of elutriator-purified cell suspension, containing 0.2×10^6 monocytes thawed and washed in RMPI-1640 medium sup-

plemented with 10% fetal calf serum (Gibco, Breda, the Netherlands), 2 mM glutamine and antibiotics, were added to 12×75-mm polypropylene tubes (Falcon Labware Division of Becton Dickinson Corp., Oxford, Calif., USA) containing 0.05 ml medium or fMetLeuPhe (final concentration of 10 nM), or with fMetLeuPhe in combination with HNscc low-M_r factors, before and after adsorption with the different antibodies (see below), or rhuIFNα2b. The tubes were incubated at 37° C in a waterbath for 15 min. The incubation was then stopped by addition of 0.25 ml ice-cold 10% formaldehyde in 0.05 M PBS (pH 7.4). The cell suspensions were kept at 4° C until counting in a haemocytometer using an ordinary light microscope (magnification 250×). The test was read "blindly" by two persons; 200 cells were counted from each tube. A cell was "polarized" if any of the following features occurred: (a) elongated or triangular shape; (b) broadened lamellipodia; (c) membrane ruffling.

The percentage of polarized monocytes was calculated as follows:

$$\frac{\text{total cells polarized (\%)}}{\text{cells positive for non-specific esterase (\%)}} \times 100$$

Lymphocytes do not exhibit any polarization activity towards fMetLeuPhe [3]. Depending on the donor, 25%–40% of the monocytes polarized in response to fMetLeuPhe (spontaneous polarization 4%–9%).

The effect of low-molecular-mass factors in sera from patients with head and neck squamous cell carcinoma and recombinant human IFNα2b on the monocyte polarization assay. Sera were collected as mentioned above and diluted 1:1 in PBS. These dilutions were subjected to ultrafiltration through Amicon CF25 Centriflo cones (Amicon Corp., Danvers, USA) for 15 min at 700 *g* (M_r "cut-off point" 25,000). The filtrates, low-M_r factors, were dissolved in PBS and stored at –20° C.

The capability of low-M_r factors or rhuIFNα2b (Intron A; Schering Corp., Kenilworth, N.J., USA) to influence the fMetLeuPhe-induced polarization of healthy donor monocytes was determined by incubating the monocytes (1×10^6/ml) for 15 min at 37° C, either with fMetLeuPhe alone, or with the tripeptide in combination with low-M_r factors (final dilution 1:60) or different concentrations of rhuIFNα2b (final concentrations 5 pM–500 nM). The percentage inhibition was calculated as follows:

$$\text{inhibition (\%)} = \left(1-\frac{P_2-P_0}{P_1-P_0}\right)\times 100$$

where P_0 = spontaneous polarization (%), P_1 = polarization after incubation with fMetLeuPhe (%) and P_2 = polarization after incubation with fMetLeuPhe and low-M_r factors or rhuIFNα.

Low-M_r factors and rhuIFNα2b were tested in triplicate, the addition of either of these to donor monocytes unstimulated by fMetLeuPhe did not affect the spontaneous polarization.

Adsorption experiments. To validate the p15E-like character of low-M_r factors, adsorption experiments were performed by neutralizing the factors before testing their effects on the monocyte polarization assay, with anti-p15E antibodies, at 4° C for 16 h and then carrying out Amicon ultrafiltration (see above) to remove the antibody-antigen complexes formed. The same procedures were performed for investigating the expression of the CKS-17 domain with an anti-CKS-17 antibody (ER-IS5) and expression of IFNα2b determinants with anti-IFNα2b antibodies. As control antibodies we used irrelevant mAb and a pAb. Antibody protein concentrations were used as shown in Table 1.

Dot-blot technique. Rauscher MuLV (≈1.0 mg protein/ml), purified as described elsewhere [29] from the JLS-V5 cell line, and rhuIFNα2b (≈0.1 mg rhuIFNα2b/ml) were applied as dots directly onto nitrocellulose strips (Bio-Rad Lab., Richmond, Calif., USA) in 5-µl volumes, and dried at 70° C for 1 h. The remaining protein-binding sites were blocked with PBS/0.05% gelatin (Gibco Europe Ltd., Paisley, Scotland) for at least 1 h. The nitrocellulose strips were then incubated overnight with anti-p15E, anti-CKS-17 and anti-IFNα2b antibodies. Bound antibodies were detected with specific conjugates, SaR-PO and RaM-PO, during a 2-h incubation step. All incubations were performed at 21° C with slow agitation, and between these steps the strips were washed three times with PBS/0.05% Tween 20 (Merck, München, Germany). The strips were developed by 0.01% H_2O_2 and 1.7 mM 4-chloro-1-naphthol (J. T. Barker B. V., Deventer, The Netherlands) in 50 mM TRIS/HCl buffer (pH 7.2), 7% polyethyleneglycol 6000 (Sigma Chemical Co., St. Louis, USA), for 5–10 min. Antibody protein concentrations were used as shown in Table 1.

Results

Effect of recombinant human IFNα2b on the monocyte polarization

Since IFNα has sequential similarities to retroviral p15E (see Introduction), we were interested in whether it was capable of inhibiting the polarization of healthy monocytes. As shown in Fig. 1, rhuIFNα2b strongly inhibited the polarization of healthy monocytes in a dose-dependent manner, with a concentration of half-maximum inhibition (IC_{50}) of about 100 nM (maximum inhibition 100%; $P_2 = P_0$, see Materials and methods). This inhibitory activity of rhuIFNα2b could not be ascribed to peptide-mediated toxicity: even with the highest concentration tested

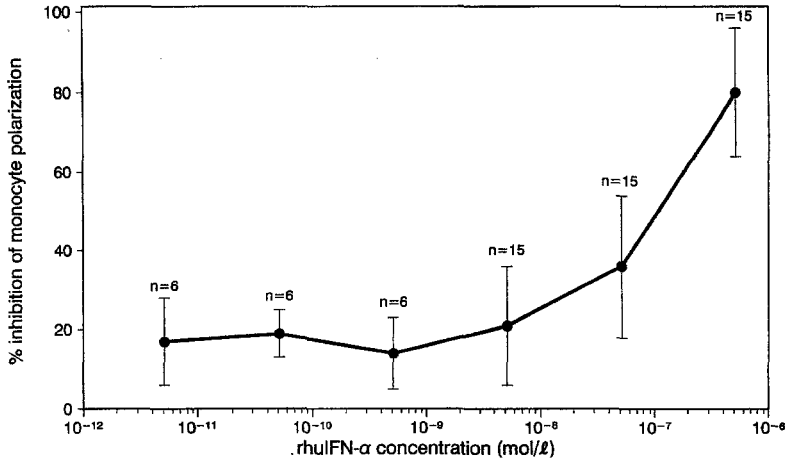

Fig. 1. The percentage inhibition of various concentrations of recombinant human interferon α2b (rhuIFNα2b) on the fMetLeuPhe-induced monocyte polarization

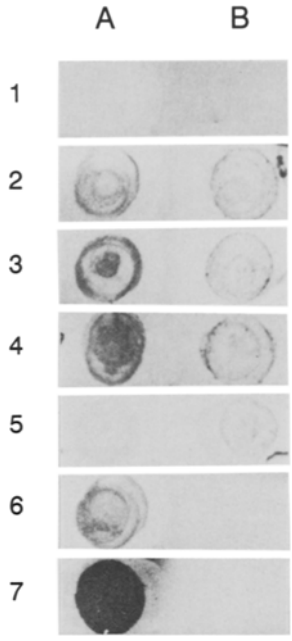

Fig. 2. Immunoreactivity (dot-blot technique) of rhuIFNα2b (*A*) and MuLV-p15E (*B*) using the following antibodies: a representative Ig control (*1*), anti-p15E antibodies 4F5 (*2*), and 19F8 (*3*), anti-CKS-17 antibodies ER-IS1 (*4*) and ER-IS5 (*5*), and anti-IFNα mAb (*6*) and pAb (*7*)

(500 nM) and incubation periods of up to 1 h, none of the monocytes showed altered morphology and an increased trypan blue uptake (data not shown).

Dot-blot technique

To study a possible structural homology between retroviral p15E and human IFNα, we also investigated whether the specific antibodies reacted with immobilized rhuIFNα2b and/or detergent-disrupted MuLV as a source of p15E, in comparison wih isotype and matched control antibodies. Figure 2 shows that anti-IFNα antibodies reacted with rhuIFNα2b, but did not show any reactivity with (dot) blotted disrupted MuLV, indicating that these antibodies showed specificity for rhuIFNα. The anti-p15E antibodies (19F8 and 4F5) reacted with both rhuIFNα2b and disrupted MuLV, indicating that these antibodies were not only specific for retroviral p15E but also crossreacted at least to a certain extent with IFNα. The anti-CKS-17 antibodies (ER-IS5 and ER-IS1) showed differential patterns of reactivity. Whereas ER-IS1 clearly reacted with both rhuIFNα2b and MuLV, ER-IS5 only very weakly reacted to disrupted MuLV.

Interestingly, the anti-p15E and anti-IFNα-specific antibodies were also able to adsorb the rhuIFNα (500 nM) inhibitory effect on the monocyte polarization (data not shown), confirming the reactivity of all these antibodies with rhuIFNα shown in the dot-blot experiments.

Reactivity of anti-p15E, anti-CKS-17 and anti-IFNα antibodies with immunosuppressive low-molecular-mass factors from the sera of patients with head and neck squamous cell carcinoma

Low-M_r factors prepared from the sera of patients with HNscc inhibit the polarization of normal monocytes [6–8]. Table 2 shows the results of adsorption experiments on the low-M_r factors prepared for serum preparations ($n = 8-11$) with the antibodies used in this study (see Table 1). The monocyte chemotaxis-inhibitory effect of all low-M_r factors tested could be adsorbed – as has been reported before – by the anti-p15E antibodies 19F8 (3 out of 3 HNscc sera tested) and 4F5 (3 out of 3) just as well as by a mixture of these two mAb (8 out of 8).

Table 2. The percentages inhibition by low-M_r factors prepared from sera of patients with head and neck squamous cell carcinoma on the fMetLeuPhe-induced monocyte polarization, before and after adsorption with antibodies directed against retroviral MuLV-p15E, its putative immunosuppressive (synthetic) CKS-17-region and rhuIFNα; the difference in inhibition of the polarization after adsorption between specific antibodies and their matching isotype controls was evaluated using the Wilcoxon's rank-sum test

Low-M_r factors	Inhibition of the monocyte polarization (%) after adsorption with									
	None	4F5/ 19F8	4F5	19F8	ER-IS5	Isotype[a] control	Anti-[b] IFNα	Isotype[c] control	Anti-[d] IFNα	Isotype[e] control
A	48	13	24	19	23	55	12	48	25	36
B	40	16	27	31	15	42	15	40	4	41
C	41	25	22	34	16	47	15	41	19	41
D	67	32	ND	ND	42	48	11	48	49	58
E	47	33	ND	ND	28	44	30	50	28	66
F	61	20	ND	ND	51	60	42	57	49	48
G	54	38	ND	ND	29	64	62	39	29	59
H	51	30	ND	ND	48	64	26	54	27	54
I	45	ND	ND	ND	ND	ND	34	ND	ND	ND
J	43	ND	ND	ND	ND	ND	20	ND	ND	ND
K	39	ND	ND	ND	ND	ND	19	ND	ND	ND
Mean	49	26	24	28	32	53	26	47	29	50
SD	9	9			14	9	15	7	15	11
P		<0.001			<0.02		<0.01		<0.02	

ND, not determined; [a] IgG2a/b; [b] mAb; [c] IgG1; [d] pAb; [e] IgGs

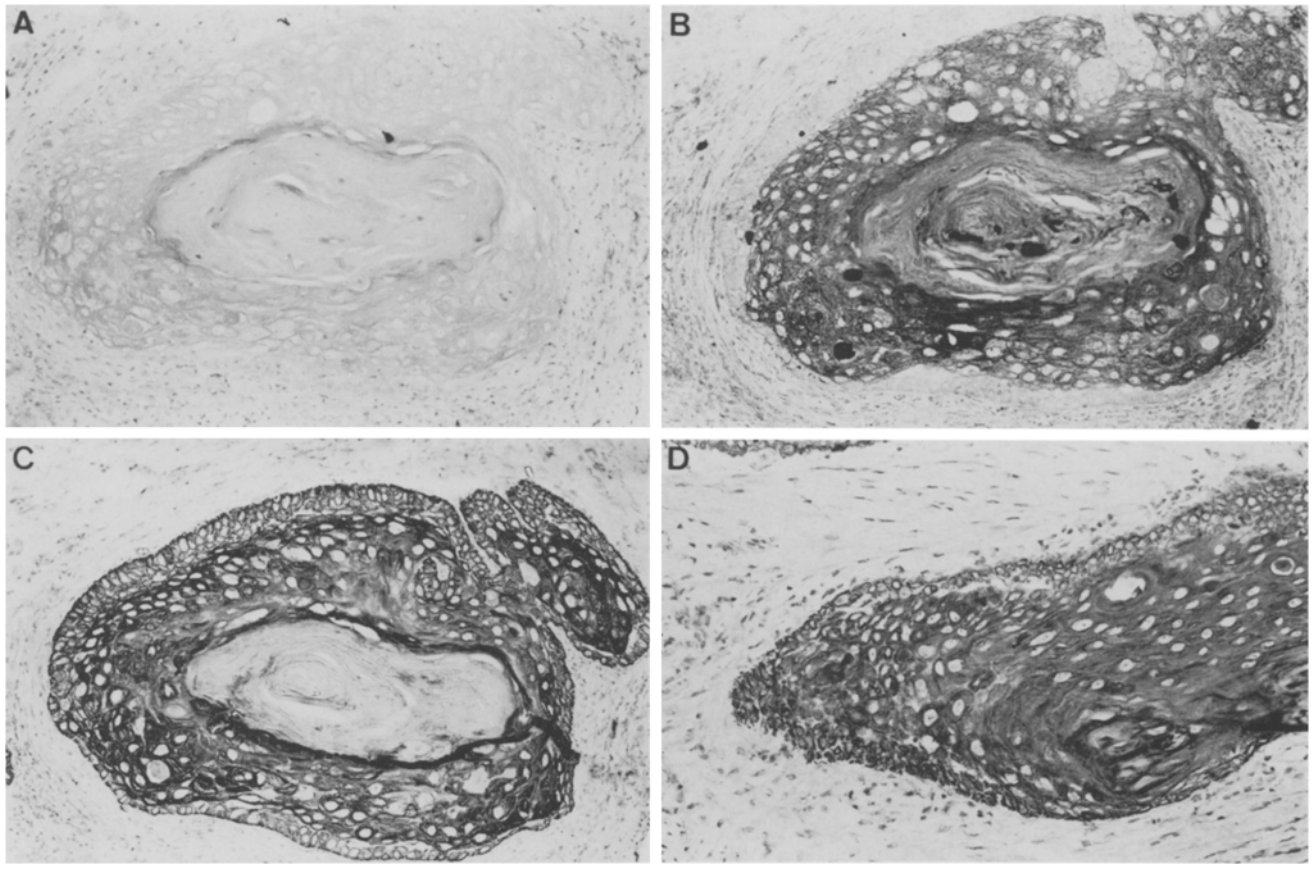

Fig. 3A–D. Immunohistochemical staining of head and neck squamous cell carcinoma specimens using the following antibodies: a representative Ig control (**A**), anti-p15E antibody 4F5 (**B**), anti-IFNα pAb (**C**) and anti-CKS-17 antibody ER-IS1 (**D**) (magnification 150×)

Interestingly, anti-IFNα2b mAb and pAb were also capable of adsorbing the inhibitory effect of the low-M_r factors. Since the anti-IFNα antibodies are specific to IFNα (dot-blot technique) and do not react with MuLV-p15E, the data of Table 2 must be taken as evidence that the majority of the low-M_r factors do contain IFNα-like molecules as immunosuppressive factors.

However, we could not find a correlation between the adsorption capabilities of the anti-IFNα antibodies and the anti-p15E antibodies, and it is thus possible that the low-M_r factors contain both p15E- and IFNα-related factors. It is also possible that different affinities of the antibodies, together with heterogeneity of the low-M_r factors, explain the non-correlation.

The relatively weak (as shown in the dot-blot experiment) anti-CKS-17 mAb ER-IS5 adsorbed the inhibitory activity of these inhibitory low-M_r factors to a certain extent (6 out of 8), indicating that these low-M_r factors express the CKS-17-like domain, as has been demonstrated previously [26].

Immunohistochemistry of head and neck squamous cell carcinoma specimens with anti-p15E, anti-CKS-17 and anti-IFNα antibodies

In accordance with our previous reports [5, 26] all (n = 18) HNscc tissue specimens studied showed a positive reactivity with both anti-retroviral p15E antibodies used, namely 19F8 and 4F5. These antibodies recognize different epitopes on retroviral p15E [27]; 19F8 is directed to an epitope in the CKS-17 domain [26]. Antibody 4F5 gave the best results in the immunohistochemistry (Fig. 3b), with practically no background staining. The positivity with the antibodies was mainly detected in corneal layers of well-differentiated tumours, and in the granular epithelial cells and prickle cells, staining the whole cytoplasm. Basal cells close to the basal membrane were often negative.

Anti-IFNα mAb and pAb also gave positive staining results with almost all (17/18) HNscc tumour specimens. However, the location of the reactivity was different from that of the anti-p15E antibodies, particularly in the case of the anti-IFNα pAb (Fig. 3c). This latter antibody clearly reacted with malignant cells in the basal epithelial cell layers close to the basal membrane, as well as with the prickle cell layers. Though this antibody was often reactive to some extent to granular cells and the corneal layers, this reactivity was not as strong as the reactivity with the anti-p15E antibodies.

The anti-CKS-17 antibodies, ER-IS5 and ER-IS1, gave staining results combining the results of the anti-p15E and anti-IFNα antibodies: in fact, all layers of the tumours were clearly reactive with these antibodies. It must be noted that ER-IS1 gave for stronger immunostaining results (Fig. 3d) than did ER-IS5, which appeared to be an antibody with low affinity (see dot-blot and adsorption experiments).

Discussion

In the present study we have shown that rhuIFNαb shares functional (monocyte polarization experiments) and structural (dot-blot technique) homology to retroviral p15E. Furthermore, our data show that low-M_r factors, prepared from the sera of HNscc patients, do not only contain factors related to retroviral p15E (as was shown extensively in earlier studies), but also factors that show antigenic similarity to IFNα. These factors appear to be separate, since there were discrepancies found in (a) the low-M_r factor adsorption data and (b) the immunohistochemistry staining results with anti-p15E and anti-IFNα antibodies.

IFNα (M_r = 19,000–26,000 [23]), well known for its antiviral actions [30], has been reported to have a wide range of immunosuppressive activities. In vivo, IFNα has been shown to decrease DTH responses in mice sensitized with sheep red blood cells [31]. In addition, the generation of antibody-producing cells was inhibited by IFNα when it was administered prior to sensitization with sheep red blood cells [32]. In vitro, IFNα is antiproliferatively active [30] and suppresses T-helper lymphocytes to stimulate antibody production by B lymphocytes [33]. Furthermore, functional suppression of monocytes/macrophages has been described: inhibition of IFNγ-induced-HLA-DR expression [34], -tumoricidal activity [35] and -respiratory burst [36] on macrophages, and blocking of IL-1-induced prostaglandin release from monocytes [37] (IL-1 inhibitor). Moreover, IFNα suppresses the in vitro maturation from human monocytes to macrophages (down-regulation of phagocytosis, complement receptors and lysosomal enzymes [38]).

In this report we complement these functional studies on IFNα by showing that rhuIFNα2b also inhibits fMet-LeuPhe-induced monocyte polarization. Furthermore, using a modified Boyden chamber assay it was found that the classical chemotaxis is suppressed by rhuIFNα2b (unpublished observation). Interestingly, the inhibitory activities on monocyte polarization of rhuIFNα2b were adsorbed by the anti-retroviral p15E antibodies. One of the latter antibodies, 19F8, recognizes an epitope within the immunosuppressive CKS-17 domain [26]. It is of particular interest that the sequence recognized by this mAb, LQNRRGLD (one-letter amino-acid code), is the sequence that is most similar to a homologous sequence within IFNα [21, 22]. This sequence has been postulated to be the minimally required sequence for immunosuppressive activity [16]. Previous studies [21, 22] have shown that the putative immunosuppressive site of retroviral p15E, CKS-17, also has IFNα-like antiviral and antiproliferative activities. Moreover, our adsorption results suggest that a sequence similar to LQNRRGLD is clearly expressed in the immunosuppressive serum factors from HNscc patients.

With regard to our finding of the IFNα-relatedness of at least some of the (HNscc sera) low-M_r factors, Sato et al. [39] have earlier found IFN activity in the sera of HNscc patients. This activity was abrogated with acid treatment. In other patient groups characterized by clear immune disorders, such as patients with systemic lupus erythematosus [40] or AIDS [41], atypical acid-labile IFNα has been demonstrated. It must be noted, however, that this acid lability was due to an acid-labile molecule, associated with, but distinct from "normal" (acid-stable) IFNα [42, 43]. In humans, it has been shown that there are at least 15 highly related IFNα subtypes [23], each with characteristic chemical and biological activities. For instance, while IFNα is generally known to be an activator of NK cell activity [30], the subtype IFNαJ lacks this ability and may even block the NK-boosting activity of other IFNα subtypes [44] (e.g. IFNαA). Hence, IFNα subtypes may exist that antagonize the immunostimulating activities of other subtypes and may even be primarily immunosuppressive.

It is tempting to suggest that the immunosuppressive factors found in HNscc are in fact related to these subtypes of IFNα, and even that p15E-related factors may be included in the IFNα family.

In conclusion, antigenic similarities observed in this study between p15E, its synthetic CKS-17 domain and human IFNα, and their known immunosuppressive action on different immunocompetent "target" cells, suggest a relationship between inhibitory HNscc p15E-related factors and human IFNα. It is likely that HNscc produce a set of heterogeneous factors that are IFNα- and/or p15E-related and exhibit inhibitory effects on monocyte chemotaxis. Isolation, purification and sequencing of HNscc immunosuppressive factors is of the utmost importance to determine whether these ill-defined factors are indeed related to the IFNα family, form a separate variety of products of (endogenous) retroviruses or represent cross-reacting activities.

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