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Depressed monocyte polarization and clustering of dendritic cells in patients with head and neck cancer: In vitro restoration of this immunosuppression by thymic hormones

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Abstract. The in vitro restoring effects of a thymic hormone preparation, TP-1, on defective monocyte and dendritic cell function in patients with head and neck squamous cell carcinoma (HNSCC) have been examined. The *N*-formylmethionyl-leucyl-phenylalanine(fMLF)-induced polarization of monocytes isolated from the peripheral blood was significantly lower (a mean of 19%) than the polarization of monocytes isolated from healthy controls (a mean of 33%). After the in vitro addition of TP-1 this defective polarization was improved to the normal value of 33% polarized monocytes. The capability of dendritic cells prepared from the blood to form cellular clusters with allogeneic cells was impaired in 26/44 patients. In vitro addition of TP-1 again had restoring effects. The original defective dendritic cell clustering of 97 clusters/six microscopic fields (mean) was improved to a value of 121 clusters. The defects in monocyte polarization and clustering of dendritic cells could be ascribed to the presence in serum of a tumor-derived low-molecular-mass factor low-M_r factor; <25 kDa) sharing structural homology with p15E, the capsular protein of murine and feline leukemogenic retroviruses. The incubation of low-M_r factor from the serum of HNSCC patients with healthy donor monocytes resulted in a significantly higher inhibition of fMLF-induced monocyte polarization than did incubation with control low-M_r factor (a mean of 42 versus 16% inhibition). This suppressive effect of patient low-M_r factor was abrogated with a mixture of two monoclonal antibodies against p15E as well as with TP-1. The observations here reported on the in vitro effects of TP-1 on depressed monocyte and dendritic cell function in HNSCC have provided one of the rationales for a TP-1 therapeutic pilot trial recently started in HNSCC patients.

Key words: Squamous cell carcinoma – Monocytes – Dendritic cells – p15E antigen – Thymostimulin – TP-1

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Introduction

Monocyte-derived macrophages and monocyte-derived dendritic cells are considered to play an important role in the immune reaction against tumor cells. Macrophages have tumoricidal activities when activated by lymphokines produced by tumor-specific T cells [17]. Dendritic cells play a role in the immune response by presenting the tumor-associated antigens to T cells. This latter phenomenon was clearly illustrated by Guyere et al. [19], who showed that effective immunity to a syngeneic sarcoma could be induced in rats by the transfer of very low numbers of dendritic lymph cells exposed to the sarcoma antigens.

Since monocytes, macrophages and dendritic cells are capable of antitumor activity it is of importance that defects in monocyte and macrophage function have been found in patients with head and neck squamous cell carcinoma (HNSCC). Balm et al. [3] found a clear impairment of the migratory capability of blood monocytes, while Cameron et al. [10] reported a defect in tumoricidal capability of macrophages. The defects in monocyte chemotaxis found by Balm et al. [3] could be ascribed to the presence in serum of a tumor-derived low-molecular-mass (low-M_r) factor (<25 kDa) capable of suppressing monocyte polarization, an early event prior to chemotaxis [43]. This immunosuppressive factor produced by the tumor shared structural homology with p15E, the capsular protein of murine and feline leukemogenic retroviruses.

Studies on defects and functioning of dendritic cells in HNSCC are scarce. Nomori et al. [35] showed that patients with a dense infiltration of these cells in nasopharyngeal carcinoma survived longer than those without such infiltration. In the present study the function of dendritic cells from the peripheral blood of patients with squamous cell carcinomas at different sites was determined. The most characteristic functional cell-biological feature of the dendritic cell is its capability actively to form cellular clusters with immunocompetent cells and create an optimal microenvironment for antigen presentation and T and B cell activation [2]. This physical association of dendritic cells with

immunocompetent neighboring cells only partly depends on antigen-specific interactions (MHC-antigen – TcR). More important are interactions between adhesion molecules on both sets of cells [23, 24, 40]. In the study reported here peripheral blood dendritic cells of HNSCC patients were allowed to form cellular clusters with allogeneic lymphocytes. An impairment of cell clustering was found in comparison to healthy controls.

We reinvestigated the defects in the chemotactic capability of monocytes isolated from the blood of patients with HNSCC [3, 44] and the presence of p15E-like factors in the patient serum [43]. In earlier studies on chronic purulent rhinosinusitis, positive for the p15E-like immunosuppressive serum factor, thymic hormone preparations (such as TP-1, Serono) abrogated the suppressive effects of the p15E-like factor on monocyte polarization [46]. Therefore, we also investigated the in vitro neutralizing effects of TP-1 on the suppression exerted by p15E-like serum factors of HNSCC patients. The in vitro restoring effects of TP-1 on the defective polarization of monocytes and the defective clustering of dendritic cells were also studied.

Materials and methods

Patients. A group of 53 patients, 38 male and 15 female, aged 37–78 years (median 59), with head and neck cancer (i.e. squamous cell carcinoma of the oral cavity, larynx and oro- and hypopharynx) of stages T1N0–T4N3 were studied.

Healthy controls. A group of 43 healthy hospital staff members, 20 male and 23 female, aged 23–54 years (median 29), volunteered as controls.

Bovine thymic extract (thymostimulin, TP-1). Thymostimulin is a bovine thymic extract prepared by Serono, according to Bergesi and Falchetti [4] and Falchetti et al. [16], using the following procedures. Calf thymus glands are minced and extracted with ammonium acetate. The extract is heated to 70° C, filtered, and precipitated with ammonium sulfate. The precipitate is dissolved in water and subjected to ultrafiltration on an Amicon PM-10 membrane. The filtrate is desalted on Sephadex G-25 and gel-filtered on Sephadex G-50. The fractions that on polyacrylamide gel electrophoresis show two characteristic bands with $R_F = 0.22$ and 0.24 are combined and termed thymostimulin (TP-1). The extract is lyophilized and its activity is expressed as units of T-cell-rosette formation per milligram of protein. It does not contain endotoxin. In toxicological studies, the extract does not cause any toxic or other side-effects in doses up to 100 mg/kg when administered to mice for 21 days or to rats for 31 days, or when administered to cats or dogs for 180 days in doses up to 50 mg/kg [16]. The extract does not alter neuromuscular transmission either in vitro or in vivo [4]. TP-1 is commercially available in Italy, Spain and Germany.

The isolation of peripheral blood monocytes. Peripheral blood mononuclear cells (from patients and healthy controls) were isolated by Ficoll/Isopaque density gradient centrifugation. The cells were washed twice in phosphate-buffered saline (PBS), pH 7.4 containing 0.5% bovine serum albumin (BSA), and counted in suspension employing positive staining with non-specific esterase [34]. The percentage of non-specific-esterase-positive cells varied from 5% to 25%. An enrichment of the monocytes in the Ficoll/Isopaque-isolated fraction was obtained by Percoll gradient centrifugation [37]: after washing, the Ficoll-isolated pellet containing both monocytes and lymphocytes was resuspended in RPMI-1640 medium supplemented with 10% fetal calf serum (Gibco, Breda, The Netherlands), 2 mM glutamine and antibiotics, and carefully underlaid with an equal volume of Percoll 1.063 (Pharmacia, Diagnostics AC, Uppsala, Sweden). After centrifugation (40 min, 400 *g*) the cells were collected from the interface, washed twice in medium (10 min, 450 *g*) and counted: the suspension now contained 70%–95% non-specific-esterase-positive cells. This suspension was directly used for the monocyte polarization and maturation of monocytes to obtain dendritic cells (see below).

Monocytes from healthy donors were isolated via counterflow elutriation centrifugation. These almost pure monocyte fractions were used to test the effects of low-M_r factor on healthy donor monocytes and dendritic cells. In brief, mononuclear cells were separated from 450 ml whole blood of the healthy controls via Percoll centrifugation (20 min, 1000 *g*, room temperature). 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 5 mg human albumin/ml. 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% monocytes as judged by positivity for nonspecific esterase activity. This fraction was used in further experiments after storage in liquid nitrogen.

Metrizamide treatment of monocytes to obtain dendritic cells. Dendritic cells were prepared from peripheral blood monocytes according to the method described by Kabel et al. [28]. Metrizamide (Serva, Heidelberg, FRG) was dissolved in RPMI medium supplemented with 10% fetal calf serum. Cells from the isolated monocytic fractions were exposed to metrizamide in suspension culture (14.5%) for 30 min (5% CO_2 and 37° C, 100% humidity). Thereafter, the cells were washed (culture fluid was added slowly to prevent osmotic lysis of the cells), and further cultured under non-adhering conditions for 16 h in polypropylene tubes (5% CO_2 and 37° C, 100% humidity). This procedure yields 40%–80% cells with a dendritic morphology, showing class II major histocompatibility complex (MHC) positivity, decreased expression of the monocytic CD14 determinant, decreased phagocytic capability, but enhanced stimulator capability in the mixed leukocyte reaction. The full technical details of this method are given in [28].

The monocyte polarization assay. The Cianciolo and Snyderman [12] assay for monocyte polarization was performed with slight modifications [44]. The assay has proven to be a rapid method to test monocyte chemotaxis and outcomes of the assay correlate well with outcomes of the conventional Boyden chamber assay to measure chemotaxis to casein [44]. Aliquots (0.2 ml) of the Percoll- or elutriator-purified cell suspension containing 0.2×10^6 monocytes were added to 12- to 75-mm polypropylene tubes (Falcon Labware Division of Becton Dickinson Co., Oxford, Calif., USA) containing 0.05 ml either medium or fMLF in medium, to reach a final concentration of 10 nM. All experiments were carried out in duplicate. The tubes were incubated at 37° C in a water bath for 15 min. The incubation was stopped by addition of 0.25 ml ice-cold 10% formaldehyde in 0.05% PBS (pH 7.2). The cell suspensions were kept at 4° C until counting in a hemocytometer 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 occurred: (a) elongated or triangular shape; (b) broadened lamellipodia; (c) membrane ruffling.

The chemotactic responsiveness of a monocyte population was expressed as the percentage of polarized monocytes in the presence of fMLF minus the percentage of polarized monocytes in the absence of fMLF. The percentage of polarized monocytes was calculated as follows:

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

Lymphocytes do not exhibit any polarization activity in this assay [18].

In samples from 77 healthy control individuals tested up till now, a mean of 33% polarized monocytes was found (SD 11%; ranges 18%–70%). There were no differences between women and men: women had a mean of 33%, SD 9% ($n = 36$); men, a mean of 32%, SD 13 ($n = 41$). Nor were differences found between individuals less than 50 years and over 50 years of age: respectively, a mean of 34%, SD 11 ($n = 66$); and a mean of 31%, SD 8 ($n = 11$). The inter-assay variation never exceeded 17% ($n = 13$); the intra-assay variation never exceeded 15% ($n = 77$).

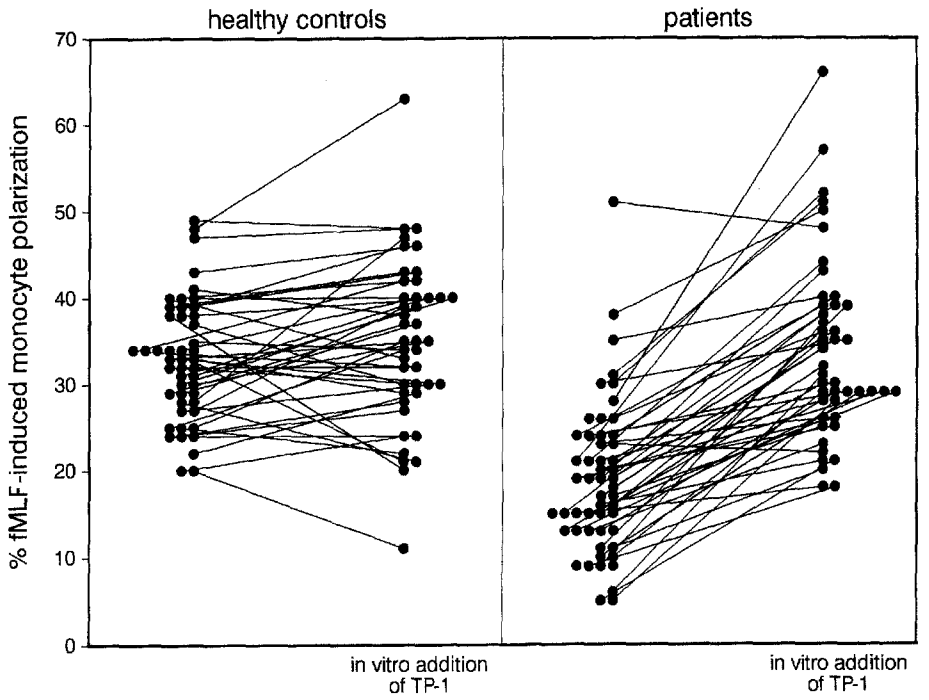

Fig. 1. The formylmethionyl-leucyl-phenylalanyl (fMLF)-induced polarization of monocytes isolated from the peripheral blood of healthy controls and head and neck squamous cell carcinoma (HNSCC) patients in the absence or presence of TP-1 (0.1 mg/ml)

Clustering of dendritic cells. The cluster assay, as described by Austyn et al. [2], was performed with modifications [28]. Samples of 5×10^4 dendritic cells, prepared from peripheral blood monocytes exposed to metrizamide, were allowed to cluster with 5×10^3 allogeneic lymphocytes isolated from healthy controls (4 h, 37° C 5% CO_2) in 250-µl flat-bottomed wells. The lymphocyte isolation was performed according to standard procedures with Ficoll/Isopaque and nylon-wool adherence (Leuko-Pak, Fenwall Laboratories, Ill., USA). Clusters formed were counted using an inverted microscope and values were expressed as the number of clusters per six microscopic fields (×200). A cluster was defined as an accumulation of 4–25 cells in tridimensional configuration.

Serum fractions. Sera were collected from the patients and healthy controls by venipuncture and diluted 1 : 1 in saline. These dilutions were subjected to ultrafiltration through Amicon CF25 Centriflo cones (Amicon Corp., Danvers, USA) for 15 min at 800 *g* (molecular mass cut-off point, 25 kDa). The residues were resuspended and stored at –20° C until further use.

Inhibition of monocyte polarization. The capability of the serum fractions to inhibit fMLF-induced polarization of healthy donor monocytes (elutriator purified) was determined by incubating the monocytes (1×10^6/ml) for 15 min at 37° C, either with fMLF alone or with fMLF in combination with a serum fraction (final dilution 1 : 60). The percentage of inhibition (i) was calculated as follows: $i(\%) = [1-(F_f-f_o/M_f-f_o)] \times 100$, where F_f = percentage monocytes polarized after incubation with fMLF and low-M_r factor, M_f = percentage monocytes polarized after incubation with fMLF alone, and f_o = percentage spontaneously polarized monocytes.

Addition of serum fractions to non-fMLF-stimulated donor monocytes did not affect the spontaneous polarization.

Adsorption experiments of low-M_r factor with mAb to p15E. To validate the p15E-like character of low-M_r factor, adsorption experiments were carried out by neutralizing the serum fractions, before testing the effects on monocyte polarization, with a combination of two p15E-specific mAb (see below) in a final dilution of 1 : 200 (final IgG concentration 50 µg/ml) at 4° C for 16 h, followed by Amicon ultrafiltration to remove formed complexes: this adsorption/neutralizing procedure was carried out twice [43]. The mAb used were a combination of 4F5 and 19F8 (anti-p15E isotypes IgG2a and IgG2b; kindly provided by Dr. G. J. Cianciolo, Genentech Inc., Pharmacological Sciences, South San Francisco, Calif., USA). As control antibodies we used irrelevant anti-human IgG2a and IgG2b.

Statistical analysis. Statistical analysis was performed by using the two-tailed Student's *t*-test, and $P < 0.05$ was taken as the level of significance.

Results

Monocyte polarization in HNSCC patients. In vitro effects of TP-1

Figure 1 shows the fMLF-induced polarization of monocytes isolated from the peripheral blood of healthy controls and HNSCC patients tested in the absence or presence of TP-1. The fMLF-induced polarization of monocytes from HNSCC patients was lower as compared to the polarization of monocytes from healthy controls: 19% (mean, SD 9, range 5–51; n = 53) versus 33% (mean, SD 7, range 20–49; n = 48); significance $P < 0.001$. This finding confirmed our previous findings on a defective chemotactic capability of monocytes from patients with HNSCC [3, 44].

After the in vitro addition of TP-1 to monocytes of HNSCC patients a restoration of the impaired function (viz., the fMLF-induced monocyte polarization) was found (Fig. 1). A dose/response effect of TP-1 was noticeable (10 patients tested, data not shown). Optimal effects were found at a concentration of 0.1 mg TP-1/ml culture fluid. At none of the concentrations tested (ranging from 0.1 µg to 10 mg TP-1/ml culture fluid) was any effect of TP-1 found on the polarization of healthy donor monocytes. Figure 1 shows the data of the effects exerted by the in vitro addition of the optimal dose of TP-1 (0.1 mg/ml) to

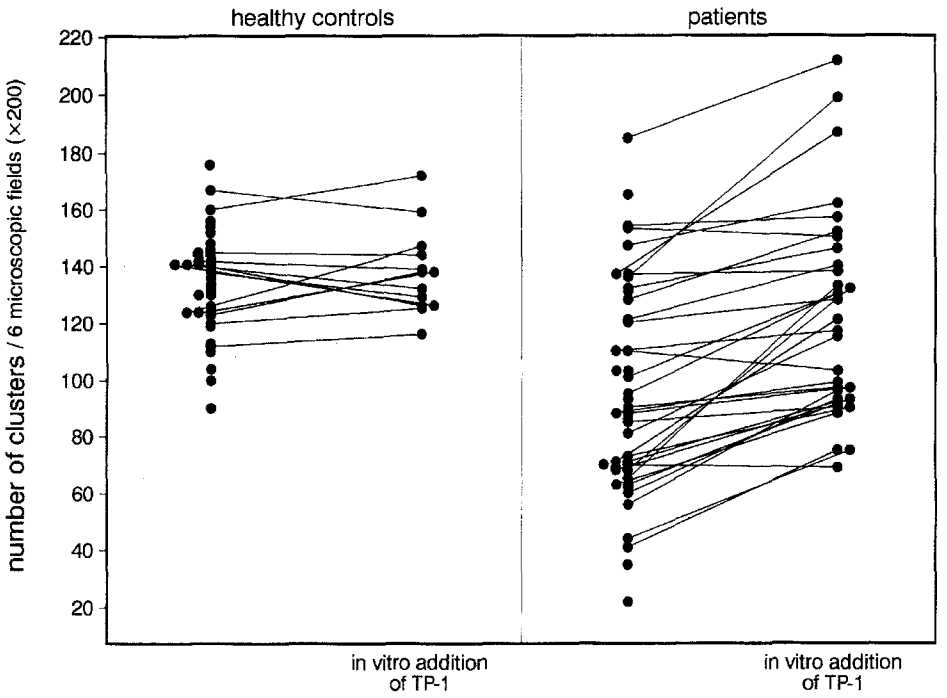

Fig. 2. The number of dendritic cell/lymphocyte clusters/six microscopic fields (×200) obtained with dendritic cells prepared from the blood of healthy controls and HNSCC patients in the absence or presence of TP-1 (0.1 mg/ml)

the monocyte suspensions obtained from a series of 45 HNSCC patients. The original defective fMLF-induced polarization of 19% polarized monocytes (mean, SD 9) was improved to the normal value of 33% polarized monocytes (mean, SD 11); significance $P < 0.0001$. TP-1 had no effect on monocyte polarization in the absence of the chemoattractant fMLF (data not shown).

Clustering capability of dendritic cells in HNSCC patients. In vitro effects of TP-1

Figure 2 shows the number of dendritic cell/lymphocyte clusters obtained after incubating dendritic cells prepared from the blood of HNSCC patients or healthy controls with allogeneic lymphocytes. The figure first shows that dendritic cells prepared from the blood of healthy controls formed 135 (mean) lymphocyte clusters/six microscopic fields (×200). The SD was 18 ($n = 39$), while outcomes ranged from 90 to 176. On the basis of these data a value of 99 clusters was considered as the lower limit of normality. In 26/44 HNSCC patients the number of dendritic cell/lymphocyte clusters formed was defective, and a mean of 94 clusters/six microscopic fields (×200) was found (SD 30); significance $P < 0.001$.

Secondly, Fig. 2 shows that the in vitro addition of TP-1 also had restoring effects on the impaired capability of dendritic cells of HNSCC patients to form cellular clusters. In a limited series of dose responses 0.1 mg TP-1/ml culture fluid again appeared to be the optimal in vitro dosage (5 patients tested; data not shown). The original defective dendritic cell clustering of 97 clusters/six microscopic fields (mean, SD 36; $n = 33$) was improved to a value of 121 clusters (mean, SD 36); significance $P < 0.0001$. In vitro addition of TP-1 to the dendritic cells of healthy controls had no effect.

Inhibition of monocyte polarization by p15E-like factors in the serum of HNSCC patients. Restoring effects of TP-1

The presence of p15E-like factors in the serum of healthy controls and HNSCC patients was determined via their biological effect, namely their suppressive effects on fMLF-induced polarization of healthy donor monocytes.

Figure 3a shows that the inhibition of fMLF-induced monocyte polarization by low-M_r factor prepared from the serum of patients with HNSCC (Fig. 3, III) was 2.6 times higher (42%; mean, SD 13; $n = 46$) than the inhibition obtained with serum low-M_r factor of healthy controls (Fig. 3, I) (16%; mean, SD 9; $n = 21$); significance <0.001. The suppressive effect of patient serum low-M_r factor was neutralizable with a mixture of two mAb against p15E (4F5 and 19F8): after adsorption of the factor with the mAb (Fig. 3, IV) values of only 19% inhibition (mean, SD 9; $n = 41$) were found; significance $p < 0.001$. Control isotype mAb did not have any effect. A small but statistically significant ($P < 0.05$) neutralizing effect of the anti-p15E was found on the small suppressive effect exerted by low-M_r factor from the healthy controls (Fig. 3, I, II).

Figure 3b shows that the in vitro addition of TP-1 to the p15E-like serum factors of HNSCC patients (VIII) was capable of neutralizing the suppressive activities of the serum factors on fMLF-induced monocyte polarization (VII) in a similar fashion to the anti-p15E (IV). In dose/response experiments the optimal concentration of TP-1 appeared to range from 67 μg/ml to 6.7 mg/ml, depending on the activity of low-M_r factor used. Since in the limited series of dose/response experiments (6 patients tested; data not shown) 67 μg TP-1/ml culture fluid always had an effect, we used this concentration in the whole series of experiments. Figure 3b shows that without the addition of TP-1 (VII) a mean of 42% inhibition of monocyte polarization (mean, SD 13; $n = 46$) was found, with 67 μg TP-1/ml

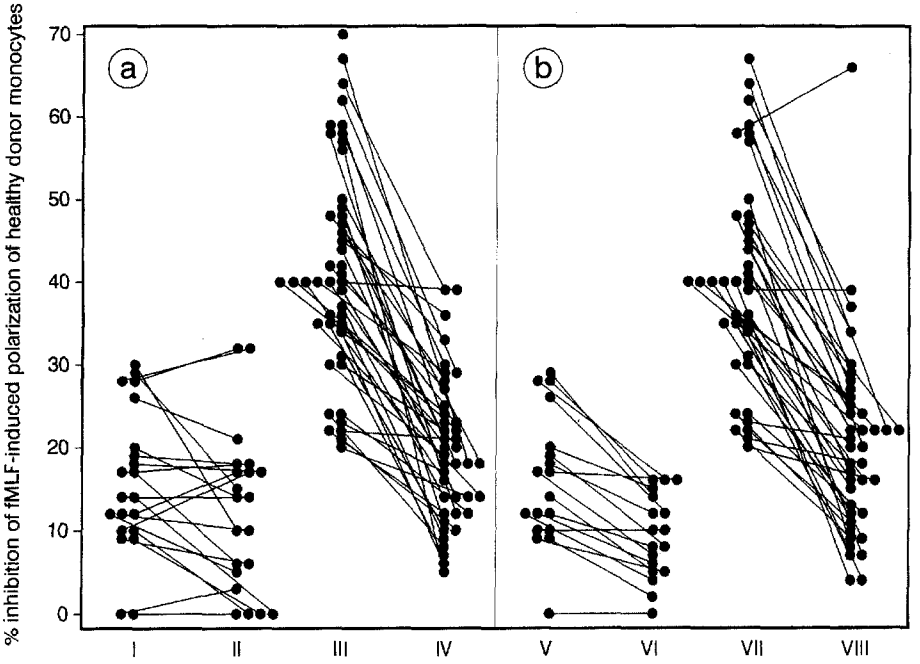

Fig. 3. The percentage of inhibition by low-M_r factor prepared from the serum of healthy controls and HNSCC patients on the fMLF-induced polarization of healthy donor monocytes, before or after neutralization with mAb to p15E (anti-p15E) (A) and in the absence or presence of TP-1 (0.067 mg/ml) (B). *I*, control low-M_r factor before adsorption with α-p15E; *II*, control low-M_r factor after adsorption with α-p15E; *III*, patient low-M_r factor before adsorption with α-p15E; *IV*, patient low-M_r factor after adsorption with α-p15E; *V*, control low-M_r factor without TP-1; *VI*, control low-M_r factor after addition of TP-1; *VII*, patient low-M_r factor without TP-1; *VIII*, patient low-M_r factor after addition of TP-1

culture fluid added to the system (VIII). Low-M_r factor-induced inhibition was practically restored to normal and values of only 20% inhibition (mean, SD 12; $n = 39$) were found (healthy controls: mean 16% inhibition, SD 9; $n = 21$); significance $P < 0.001$. The addition of TP-1 to factor from healthy donor serum (VI) had a small but statistically significant effect (without TP-1 mean 16%, SD 9; $n = 21$, versus with TP-1 mean 9%, SD 5; $n = 18$).

Discussion

In the present study we show a defect in the clustering of dendritic cells, matured from the peripheral blood monocytes of HNSCC patients and confirm our earlier reports on a defective monocyte chemotaxis in HNSCC patients (as measured in the polarization assay) and the presence in patient serum of low-M_r factor related to retroviral p15E capable of suppressing monocyte chemotaxis [3, 43, 44].

The defect in monocyte chemotaxis in patients with HNSCC has also been confirmed by others [49]. These authors also found that the number of formylpeptide binding sites on HNSCC patient monocytes was increased despite their defective function. Bugelski et al. [8, 9] have reported that in several animal models (the 13762NF MTLn3 rat mammary adenocarcinoma, the B16-BL6 mouse melanoma, the M5076 mouse reticulum cell sarcoma and the autochthonous reticulum cell sarcomas in SJL/J mice) the recruitment of monocytes/macrophages into primary sites or metastases is decreased as tumor mass or the metastases enlarge. This results in a decrease in the density of tumor-infiltrated macrophages. Whether our findings of a disturbed polarization of blood monocytes in HNSCC patients also results in a decrease in the density of human infiltrated macrophages and dendritic cells into the tumor site is presently being investigated. The defects in clustering of peripheral blood dendritic cells found in HNSCC patients reported here are in line with earlier findings of disturbed cell-mediated immune functions, in which these cells play a role. There are numerous reports on poor responsiveness to DNCB sensitization in HNSCC patients [38, 6], low numbers of peripheral T cells [27, 31] and diminished in vitro lymphocyte mitogenic responses [6, 11]. With regard to the presence of dendritic cells in HNSCC, Nomori et al. [35] showed that patients with a dense infiltration of these cells in primary sites survived longer than those without such infiltration. It is noteworthy that there was no such relationship between prognosis and density of lysosome-positive macrophages at the tumor site. This might indicate that the role of dendritic cells in tumor defence is more important than the role of tumor-infiltrating macrophages. This view is further supported by the observations of Schroder et al. [42] and Bröcker et al. [7] on the inflammatory cell infiltrates in human papillary thyroid carcinomas and melanomas respectively. In their studies the determination of the quantity of dendritic cells at the tumor site also proved to be a highly effective means of assessing the prognosis of such tumors. On the other hand, the degree of infiltration of macrophages and lymphocytes was not associated with a distinct biological behavior. Whether the defective clustering capability of peripheral blood dendritic cells in HNSCC patients correlates with their defective infiltration and/or defective function in the tumor itself is also presently being investigated.

Changes in the number and morphology of locally infiltrated dendritic cells in response to tumor growth have extensively been studied by Bergfelt et al. [5]. These authors showed that the changes found were probably due to a malignant factor released by the tumor, which they could not identify. A tumor factor influencing monocyte chemotaxis has been known since 1981. In that year it was demonstrated by Cianciolo et al. [13] that the inhibitory effect on monocyte chemotaxis was due to low-M_r factor

produced by both animal and human malignant cells and that this effect could be adsorbed by any of three different mAb to the retroviral capsular protein, p15E. The presence of these p15E-like factors in HNSCC patients and the suppressive effects on monocyte function have later been described by Balm et al. [3] and Tan et al. [44]. The present findings support these earlier reports. It is known that the suppressive effects of p15E-like factors and a 17-amino-acid peptide synthesized from the highly conserved region of MuLV p15E (CKS-17) are not restricted to monocyte chemotaxis, but also have suppressive effects on monocyte-mediated killing by inactivating interleukin-1 (IL-1) [29, 18], on the respiratory burst of human monocytes [20], on feline neutrophil activation [30], on the IL-2- or IL-1-dependent proliferation and the blastogenic responses to mitogens and allo-antigens of T cells [14, 32, 36, 39, 41] on the human natural killer cell activity of NK cells [21], on polyclonal B cell activation [33] and, more relevant to this report, on the clustering capability of dendritic cells [47]. It is likely that HNSCC-derived p15E-like factors are also responsible for the impaired clustering capability of the HNSCC dendritic cells shown in the present report. In a few experiments it was indeed found that the serum p15E-like factor of HNSCC patients suppressed the clustering capability of healthy donor dendritic cells (data not shown). With regard to this latter effect, it must be noted, however, that p15E-like factors seem to exert a suppression on the clustering capability of dendritic cells that is less marked than the suppression exerted on monocyte polarization [47].

The presence of p15E-like factors in serum is not specific for malignant disease. In Graves' disease [47] and chronic purulent rhinosinusitis [48] the presence of p15E-like factors in serum has also been described. Moreover, p15E-like factors are detectable by immunohistochemical techniques in thymic epithelial cells, epithelial cells overlaying areas of inflammation [45], monocytes (unpublished observations, Tas et al.) and p15E-like factors are produced in macrophages after glucocorticosteroid treatment [22]. This suggests that the p15E-like factors are physiological regulators of immune reactivity. Therefore, it is of particular interest that in healthy controls a small activity of low-M_r factor on monocyte polarization was found and that this immunosuppressive activity could partly be adsorbed by the thymic hormone preparation, TP-1, or mAb directed against p15E. In patients with chronic purulent rhinosinusitis, treatment with TP-1 resulted in a abrogation of the suppressive effects of the p15E-like serum factor [46]. In the present study we showed the in vitro restoring effects of TP-1 on defective monocyte polarization and defective clustering of dendritic cells. It is likely that TP-1 produced these effects by counteracting the suppressive effects of the p15E-like factors, since in direct competition experiments TP-1 abolished the effects of p15E. Thymic hormones are known to have strong effects on the functioning of the cell-mediated immune system. In neonates they induce a terminal differentiation in T cells, and are able to stimulate T-lymphocytes to produce lymphokines [1, 16]. In adults the administration of thymic hormones has also been described as effective; and lowered levels of endogenous thymic hormones can be found in several secondary immunodeficiency states, in viral infections and other states of impaired adult host defence [25, 26]. Beneficial outcomes of thymic hormone treatment in these conditions are generally attributed to a direct effect on T cell maturation. From our data it is, however, tempting to speculate that other mechanisms may be involved as well and that these thymic factors may exert, at least in part, their effect via cells of the monocyte/macrophage/dendritic cell system and via the neutralization of an endogenously produced immunosuppressive factor. Such an effect of thymic hormones could explain their beneficial effects in adults with malignant growth and cell-mediated immunodeficiencies in the presence of a full T cell repertoire [15].

The observations reported here on the in vitro effects of TP-1 on depressed monocyte and dendritic cell function in HNSCC has provided one of the rationales for a recently started TP-1 therapeutic pilot trial in HNSCC patients.

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