Thymomodulin increases HLA-DR expression by macrophages but not T-lymphocyte proliferation in autologous mixed leucocyte reaction


ABSTRACT: Thymomodulin (TMD), a thymic biological response modifier, stimulates the release of tumour necrosis factor (TNF) and granulocyte-macrophage-colony stimulating factor (GM-CSF) in macrophage-lymphocyte cultures. We investigated the effects of the cytokines released in cultures with TMD, on the expression of human leucocyte antigen-DR (HLA-DR) antigens by alveolar macrophages (AM) and the T-cell proliferation induced in autologous mixed leucocyte reaction (AMLR) cultures or by T-cell mitogens.

Among freshly isolated AM, 84±4% were HLA-DR positive, and this proportion was significantly reduced after 24 h cultures (60±3%, p<0.05). In cultures without peripheral blood (PBL) lymphocytes, TMD did not change HLA-DR expression by AM (HLA-DR+AM); whilst in the presence of autologous PBL lymphocytes, TMD induced an increase in the proportions of HLA-DR+AM (TMD 100 µg·ml−1 79±3%, p<0.04 vs control cultures). However, TMD did not change the ability of AM to induce T-cell proliferation in AMLR between AM and PBL lymphocytes. In contrast, in PBL mononuclear cell cultures, TMD induced a further increase of the cell proliferation due to the T-cell mitogens interleukin-2 (IL-2) or phytohaemagglutinin (PHA) (p<0.05 vs each control culture with mitogens) or anti-CD3 antibodies (p<0.03 vs control cultures).

Thus, the cytokines released in cultures with TMD enhance macrophage HLA-DR expression. Whilst this phenomenon is not associated with changes in the ability of AM to stimulate T-cell proliferation, TMD is able to increase the mitogen-induced T-cell proliferation.


Investigating the effects of thymomodulin, a thymic biological response modifier, on cultures of alveolar macrophages and of blood lymphocytes, we have previously demonstrated that thymomodulin increases the release of tumour necrosis factor (TNF) and of granulocyte-macrophage-colony stimulating factor (GM-CSF) in co-cultures of macrophages with autologous lymphocytes, and stimulates GM-CSF production by lymphocytes [1]. Since the cytokines modulate the activities of inflammatory and immunocompetent cells, the ability of thymomodulin to stimulate the release of TNF and GM-CSF suggested that it could have other effects on macrophage and lymphocyte functions. In the lung, the interaction between macrophages and lymphocytes, involving cytokine release, expression of surface molecules and cellular proliferation, is a basic step of each immune reaction to antigenic stimuli [2, 3]. In this context, TNF and GM-CSF, among their many activities, are able to modulate the expression of surface molecules, including human leucocyte antigen DR (HLA-DR) molecules [4-8]. The expression of the HLA-DR surface molecules by alveolar macrophages is a necessary prerequisite in the process of antigen presentation to T-lymphocytes, followed by the proliferation of antigen specific T-cells [2, 3, 9].

With this background, the present study was designed to determine whether thymomodulin is able to modulate the expression of HLA-DR molecules on macrophages in human alveolar macrophage and blood lymphocyte cultures. In addition, the ability of T-cells to proliferate in autologous mixed leucocyte reaction (AMLR) with alveolar macrophages, or after T-cell mitogen stimulation was tested. The data demonstrate that thymomodulin induces an increase in macrophage HLA-DR expression. However, thymomodulin does not change T-cell proliferation in AMLR, but further increases the T-cell mitogen-dependent T-cell proliferation.
Materials and methods

Study population

Twenty two individuals (12 males and 10 females, 28–60 yrs of age), referred to the First Division of Lung Diseases, San Martino Hospital, Genoa, Italy, entered this study. These individuals underwent fibroptic bronchoscopy as part of the evaluation for respiratory symptoms, and/or for chest X-ray abnormalities and were chosen according to the following criteria: 1) nonsmokers or former smokers who quit smoking at least one year before; 2) taking no medication at the time of the study; 3) no known exposure to industrial or noxious pollutants; 4) results of lung function tests (total lung capacity (TLC), vital capacity (VC), forced expiratory volume in one second (FEV),), diffusing capacity of the lungs for carbon-monoxide (DLCO) within normal values. The final diagnosis of these individuals was: adenocarcinoma of the lung in three cases, small cell carcinoma of the lung in three cases, epidermoid carcinoma of the lung in four cases, localized metastatic lung involvement from tumours arising in other organs in three cases and tuberculosis in one case, whilst in eight cases no evidence of bronchial or lung disease was found. Five normal volunteers were used to obtain only peripheral blood non-adherent cells. Informed consent was obtained before venipuncture and bronchoalveolar lavage (BAL) procedures.

Collection of lung and blood mononuclear cells

Cells were obtained from the lower respiratory tract of all 22 patients by means of BAL, which was performed in all subjects in areas free of any visible inflammatory or neoplastic endoscopic lesion, using a total of 100 ml in five 20 ml aliquots of 0.9% sterile saline solution, as described previously [1, 2]. After centrifugation, determination of viability, total and differential cell counts, the cells were resuspended at the desired cell density in the appropriate medium (see cell culture sections).

Heparinized blood samples were obtained by means of venipuncture in patients undergoing fibroptic bronchoscopy, and in five normal volunteers. Peripheral blood mononuclear cells were isolated by Ficoll density gradient [2], washed several times in Hank's balanced salt solution (HBSS) without Ca++ and Mg++, resuspended in RPMI-1640 (Flow Laboratories, Irvine, Scotland, UK), with 10% autologous plasma, and counted. Peripheral blood mononuclear cells from patients undergoing bronchoscopy were enriched for T-lymphocytes by adherence onto culture dishes followed by washing medium at 4°C, counted, and finally resuspended in normal volunteers were cultured without T-cell purification, as described below.

Thymomodulin

Thymomodulin (Ellem, Corso di Porta Ticinese, 89, Milano, Italy) is derived from calf thymus by acid lysis filtration and lyophilization. The result is a mixture of peptides, MW less than 10,000 Da. In vitro and in vivo studies have shown that thymomodulin is able to regulate the maturation of T-lymphocytes, to act on functional activities of mature B- and T-lymphocytes and other inflammatory cells [10–17].

Evaluation of the expression of surface HLA-DR molecules by alveolar macrophages

In order to evaluate the percentages of alveolar macrophages expressing HLA-DR surface molecules, macrophages immediately after BAL (fresh macrophages) and macrophages after 24 h cultures (cultured macrophages) were stained with the L243 monoclonal antibody (Becton Dickinson), recognizing a non-polymorphic determinant on HLA-DR molecules [18, 19]. Macrophage culture conditions were as described previously [1]. Briefly, alveolar macrophages obtained by means of BAL were cultured in RPMI-1640, 5% heat-inactivated foetal bovine serum (FBS) (Flow laboratories), glutamine 20 mM, penicillin 100 U·ml⁻¹ and streptomycin 100 μg·ml⁻¹ in 1 ml final volume, in 24 well plates, for 24 h with or without γ-interferon (γ-IFN) (100 U·ml⁻¹) or thymomodulin at various concentrations (1, 10, 100 μg·ml⁻¹). HLA-DR expression data were obtained from cultures of macrophages (3×10⁶ cells·ml⁻¹) from eight patients (five patients with neoplastic lung involvement, three patients without lung tumours), or from co-cultures of macrophages (3×10⁶ cells·ml⁻¹), obtained from nine patients (six from patients with neoplastic lung involvement, three from individuals without lung neoplasm), with autologous blood lymphocytes (1×10⁶ cells·ml⁻¹), containing >93% CD2+ T-lymphocytes. The levels of various cytokines were tested in the supernatants of these cultures [1].

Fresh or cultured macrophages (detached from the culture wells by gentle, repeated resuspension) were incubated at 4°C for 20 min in phosphate buffered saline (PBS), pH 7.4, supplemented with 10% normal human AB serum, to reduce the binding of staining antibodies with macrophage Fc receptors. Cells were then washed twice at 4°C in washing medium (PBS 1% FBS) and resuspended in 20 μl of washing medium; 10 μl of the control, isotype matched, nonrelevant mouse antibody fluorescein isothiocyanate (FITC)-conjugated (Becton Dickinson) or of the L243 anti-HLA-DR FITC-conjugated monoclonal antibody were then added. After 20 min at 4°C, cells were washed three times with washing medium at 4°C, fixed in 1% paraformaldehyde for 30 min, and finally...
resuspended in 0.5% paraformaldehyde until fluorescence-activated cell sorter (FACS) analysis was performed. The cells were analysed with a FACS analyser (Becton Dickinson). The data were collected and stored in list mode files and analysed using a Consort 30 software (Becton Dickinson). Macrophages were discriminated from lymphocytes according to the characteristic scatter profile on FACS analysis [20]. To reduce the background of autofluorescence, the FACS photomultipliers were set so that macrophage autofluorescence in each control sample was in the fluorescence range of 10^4. The proportions of macrophages staining positive were calculated by subtracting the control sample value from the value obtained using the anti-HLA-DR antibody. To better compare results obtained on different days, the logarithmic amplifiers were calibrated using fluorescent beads (Immunosure, Coulter Immunology, Hialeah, FL, USA; Fluortrel-GF, Ortho Diagnostic Systems, Raritan, NJ, USA) and the analysis was performed on identical settings.

**Evaluation of the ability of thymomodulin to stimulate T-cell proliferation in AMLR**

Since macrophages, like other antigen presenting cells, interact with the T-cell antigen receptors on T-lymphocytes through the HLA-DR surface molecules, inducing T-cell activation and proliferation [2, 3, 21], we determined whether, in AMLR between alveolar macrophages and blood lymphocytes, thymomodulin was able to induce changes in the in vitro proliferation of T-cells. BAL cell suspensions from five patients undergoing fibrocapsic bronchoscopy (two patients with lung cancer and three patients without neoplastic lung involvement) containing >93% alveolar macrophages, with a viability of >95%, (trypan blue exclusion) were used. BAL cells were washed twice and resuspended in complete medium (RPMI-1640 supplemented with glutamine 20 mM, 100 U·ml⁻¹ penicillin, 100 µg·ml⁻¹ streptomycin, 5% FBS and 5 mM 2-mecaptopethanol). Autologous peripheral blood was used as a source of T-lymphocytes. T-cells were partially purified, as described above, by adherence. The final lymphocyte suspensions, containing >93% CD2+ T-lymphocytes, were resuspended in complete medium. Cultures of alveolar macrophages (AMs) alone or of peripheral blood lymphocytes (PBLs) alone or AMLR cultures of macrophages (previously irradiated with 3,000 rads) with lymphocytes were performed in duplicate, with or without thymomodulin (1, 10 and 100 µg·ml⁻¹ final concentration), in 24 well plates (Flow Laboratories, Milano, Italy), at 37°C in 5% CO₂. In AMLR cultures, 2.5x10⁵ AMs were present in each well; since the stimulatory capacity of antigen presenting cells is dependent upon their numbers [2, 3], graded numbers of lymphocytes were added to each well. Maximal response was observed with a blood lymphocyte to alveolar macrophage ratio of 4:1, and the results shown refer to these data.

Proliferation was assessed on day 6, after the addition of 20 µCi-well⁻¹ of tritiated thymidine (HTdR) (specific activity 20 µCi·mmol⁻¹) (New England Nuclear, Boston, MA, USA) for the last 18 h of culture. The cells were harvested with a Skatron (Sterling, VA, USA) cell harvester, and T-cell proliferation was assessed evaluating the HTdR incorporation using a β-counter (1209 Rakketa, LKB Wallace, Turku, Finland).

**Evaluation of the ability of thymomodulin to stimulate T-cell proliferation in mitogen-driven peripheral blood mononuclear cell cultures**

Since most of the known effects of thymomodulin are on lymphocytes [1, 10–12], we tested the effects of the compound on blood mononuclear cells cultured with different T-cell mitogens. Peripheral blood mononuclear cells (5x10⁶), isolated by means of Ficoll centrifugation of heparinized peripheral blood from five normal individuals, were cultured in complete medium in 96 well plates (Flow Laboratories, Milano, Italy) for 4 days at 37°C, in 5% CO₂. Cultures were performed with or without: 30 U·mi⁻¹ of recombinant human interleukin-2 (IL-2) (Cetus, Emeryville, CA, USA), 0.1 µg·ml⁻¹ of anti-CD3 monoclonal antibodies (Ortho Diagnostic, Milano, Italy) and 1 µg·ml⁻¹ of phytohaemagglutinin (PHA). In addition, all the above cultures were performed in the presence or absence of thymomodulin (1, 10 and 100 µg·ml⁻¹). After 3 days in culture, HTdR was added to each well for the last 18 h and incorporated radioactivity was detected as described above.

**Statistical analysis**

All data are presented as mean±standard error of the mean; statistical analysis for multiple comparisons was made with the analysis of variance (ANOVA) test and the two-tailed Student’s t-test. The mean values of various parameters were said to be significantly different when the probability of the differences of that magnitude, assuming the null hypothesis to be correct, fell below 5% (i.e. p<0.05).

**Results**

**Characterisation of cell populations recovered by lavage**

Bronchoalveolar lavage was performed in all subjects without complications. Visualisation of the bronchial tree in the lavaged areas demonstrated no evidence of inflammatory or neoplastic lesions. The recovered fluid was sterile in all cases. The total amount of fluid recovered for each 100 ml of saline infused was 66±5 ml, and the total cells recovered were 11.4±1.9x10⁶, comprised of 90±2% macrophages,
6±2% lymphocytes and 1.2±0.4% neutrophils or eosinophils. There were no differences in the amount of fluid recovered or the total and differential cell counts, between the patients with primary or metastatic lung tumours or without neoplastic lung involvement (p>0.2 each comparison) (table 1).

Macrophage HLA-DR expression immediately after bronchoscopy and after 24 h cultures with or without thymomodulin

Evaluation of surface HLA-DR molecules of AMs recovered by BAL from patients undergoing bronchoscopy showed that 84±4% of the cells reacted with the anti-HLA-DR monoclonal antibody L243 (figs 1 and 2).

In this context, comparing samples from patients with neoplastic lung involvement to those without neoplastic disease, no difference was found for the proportions of HLA-DR positive macrophages (84±4 vs 84±3%, p>0.5).

When macrophages were cultured in control medium alone, without stimuli for 24 h, 62±4% of the cells were HLA-DR positive, a percentage significantly lower than that observed in fresh macrophages (p<0.05) (fig. 1). In contrast, when alveolar macrophages were cultured for the same amount of time in the presence of γ-IFN, 91±2% of the cells were HLA-DR positive, a value significantly higher when compared to unstimulated cultured cells (p<0.03) (fig. 1). When 1, 10 and 100 μg·ml⁻¹ of thymomodulin were added to the macrophage cultures, no significant

| Table 1. Bronchoalveolar lavage data from patients with and without neoplastic lung involvement |
|---------------------------------------------|-----------------|---------------|---------------|---------------|
| Fluid recovered | Cells | % Mac | % Lymph | % PMN |
| Patients with cancer (n=13)* | 65±3 | 11.0±1.4 | 91±2 | 6±2 | 1.3±0.3 |
| Patients without cancer (n=9)* | 66±4 | 11.5±1.8 | 90±2 | 7±2 | 1.1±0.4 |

1: per 100 ml infused; *: see methods and results for diagnosis. p>0.2 each comparison between the two patient groups. Mac: macrophage; Lymph: lymphocytes; PMN: polymorphonuclear leucocytes.

Fig. 1. – Proportions of HLA-DR positive alveolar macrophages fresh and cultured with or without γ-interferon or different concentrations of thymomodulin. Alveolar macrophages obtained from eight patients undergoing fibreoptic bronchoscopy were stained with the monoclonal antibody L243 (Becton Dickinson) specific for HLA-DR molecules, using direct immunofluorescence and analysed by flow cytometry. Fresh: values obtained staining alveolar macrophages immediately after bronchoalveolar lavage. All other values refer to macrophages cultured to 24 h with the following stimuli: C: control medium; γ-IFN; γ-interferon, 100 U·ml⁻¹; TMD 1, 10 and 100: thymomodulin 1, 10 and 100 μg·ml⁻¹. AMs: alveolar macrophages; HLA-DR: human leucocyte antigen DR.

Fig. 2. – Proportions of HLA-DR positive alveolar macrophages fresh and cultured in the presence of autologous peripheral blood lymphocytes with or without γ-interferon or different concentrations of thymomodulin. Alveolar macrophages obtained from nine patients undergoing fibreoptic bronchoscopy were stained with the monoclonal antibody L243 (Becton Dickinson) specific for HLA-DR molecules, using direct immunofluorescence and analysed by flow cytometry. Fresh: values obtained staining alveolar macrophages immediately after bronchoalveolar lavage. All other values refer to macrophages cultured for 24 h with the following stimuli. For abbreviations see legend to figure 1.
change in the percentages of HLA-DR positive alveolar macrophages was observed, as compared to unstimulated macrophages (p>0.5 each comparison) (fig. 1).

Similarly, when alveolar macrophages were cultured in control medium without stimuli with autologous peripheral blood non-adherent cells for 24 h, the proportions of HLA-DR positive macrophages decreased significantly, as compared to fresh alveolar macrophages (60±3 vs 84±4%, p<0.05) (fig. 2). The addition of γ-IFN to the macrophage-lymphocyte cultures increased, after 24 h, the proportions of HLA-DR positive macrophages (88±3%, p<0.04 with 24 h macrophage-lymphocyte cultures without stimuli) (fig. 2). The addition of thymomodulin at the concentration of 1 and 10 μg·ml⁻¹ did not change the percentages of HLA-DR positive macrophages after 24 h cultures (p>0.5 with control cultures) (fig. 2).

In contrast, flow cytometric analysis clearly demonstrated that in cultures of macrophages and autologous blood lymphocytes, the addition of 100 μg·ml⁻¹ of thymomodulin was associated with an increase in the proportions of HLA-DR positive macrophages (79±3%, p<0.04 with control cultures) (fig. 2). Figure 3 depicts an example of flow cytometric analysis of HLA-DR expression by cultured macrophages. In this example, alveolar macrophages, obtained through bronchoalveolar lavage from a patient with lung cancer, were cultured with autologous peripheral blood lymphocytes in complete medium, without (Panel A) or with the addition of thymomodulin 100 μg·ml⁻¹ (Panel B). Note the increased proportion of HLA-DR positive macrophages in the culture with thymomodulin (Panel B), as compared with macrophages cultured without thymomodulin (Panel A).

### Table 2. Proportions of HLA-DR positive alveolar macrophages in cultures with autologous peripheral blood lymphocytes from patients with and without neoplastic lung involvement

<table>
<thead>
<tr>
<th>Stimuli</th>
<th>Control</th>
<th>TMD 1</th>
<th>TMD 10</th>
<th>TMD 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with cancer (n=6)*</td>
<td>61±3</td>
<td>57±4</td>
<td>65±4</td>
<td>79±3</td>
</tr>
<tr>
<td>Patients without cancer (n=3)*</td>
<td>59±2</td>
<td>58±3</td>
<td>64±4</td>
<td>78±2</td>
</tr>
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*: see methods and results for diagnosis. HLA-DR positive alveolar macrophages are expressed as percentages of total alveolar macrophages. p>0.2 each comparison between two patient groups. TMD 1, 10 and 100: thymomodulin, 1, 10 and 100 μg·ml⁻¹; HLA-DR: human leucocyte antigen.

Throughout the study, no difference was seen between patients with or without neoplastic lung involvement in the proportions of macrophages cultured alone or with autologous lymphocytes (table 2) reacting with the anti-HLA-DR antibody (p>0.2, each comparison).

### Ability of thymomodulin to stimulate in vitro T-cell proliferation in AMLR

In mixed leucocyte reaction (MLR) cultures, HLA-DR positive antigen presenting cells induce the in vitro proliferation of T-lymphocytes [2, 3]. In our experiments AMs, as antigen presenting cells, were able to induce the proliferation of autologous blood T-cells (fig. 4). In this context, PBLs cultured alone showed a minimal incorporation of [³H]Tdr (180±32 cpm) (fig. 4). Similarly, AMs showed no significant proliferation when cultured alone (125±15 cpm) (fig. 4).
When irradiated AMs were co-cultured with purified autologous blood lymphocytes, there was a clear stimulation of T-cell proliferation (5.950±580 cpm, p<0.01 each comparison with macrophages alone and with lymphocytes alone) (fig. 4). The addition to the MLR cultures, as well as to the control cultures of macrophages alone or of lymphocytes alone, of 1, 10 and 100 µg·ml⁻¹ of thymomodulin, was not associated with any significant change in ³HThdr incorporation (p>0.5 each comparison) (fig. 4). Thus, despite the ability of thymomodulin to induce a higher HLA-DR expression by AMs, the addition of thymomodulin to MLRs did not increase the ability of macrophages to stimulate T-cell proliferation.

Finally, although the patient population participating in the MLR experiments included two patients with neoplastic lung involvement, cell proliferation data from the patients without lung cancer were similar to the data obtained grouping all patients. In this regard, MLR cultures of cells from the non-neoplastic patients had an incorporation of 5.800±550 cpm, that was not significantly changed in MLR cultures with thymomodulin (p>0.2) (not shown).

**Ability of thymomodulin to stimulate in vitro T-cell proliferation in mitogen-driven T-cell cultures**

In T-lymphocyte cultures with T-cell mitogens, the cell proliferation is basically dependent upon the ability of T-cells to be directly activated through different surface molecules. The addition of recombinant IL-2, of anti-CD3 antibodies and of PHA to cultures of peripheral blood mononuclear cells was associated with increased T-cell proliferation (control cultures: 0.4±0.2 cpm x10⁶, cultures with IL-2 1.8±0.5 cpm x10⁶, cultures with anti-CD3 2.1±0.3 cpm x10⁶, cultures with PHA 22±2 cpm x10⁶, p<0.05 each comparison) (table 3). Thymomodulin at 1 µg·ml⁻¹ was able to increase the T-cell proliferation induced by the addition of recombinant IL-2 (cultures with IL-2 and with thymomodulin 2.9±0.2 cpm x10⁶, p<0.05 with cultures with IL-2 alone) and that stimulated by anti-CD3 antibodies (cultures with anti-CD3 and with thymomodulin 3.6±0.3 cpm x10⁶ p<0.03 with cultures with anti-CD3 alone) (table 3). Similarly, in cultures of non-adherent cells stimulated with PHA the addition of 10 µg·ml⁻¹ of thymomodulin was associated with increased cell proliferation (cultures with PHA and with thymomodulin 10 µg·ml⁻¹ 37±4 cpm x10⁶, p<0.05 with cultures with PHA alone). At higher thymomodulin concentrations this effect on T-cell proliferation was plateauing for all the tested mitogens (table 3).

**Discussion**

We studied the *in vitro* effects of a calf thymus derivative, thymomodulin, on alveolar macrophages and blood lymphocytes and demonstrated that thymomodulin: 1) increases the HLA-DR expression by macrophages in macrophage-lymphocyte cultures; 2) does not influence the ability of macrophages to induce autologous T-cell proliferation in mixed leucocyte reaction; and 3) increases the T-cell proliferation induced by T-cell mitogens in blood mononuclear cell cultures.

In a study on the effects of thymomodulin on cytokine production by macrophages and lymphocytes, we demonstrated that thymomodulin is able to induce an increase of the TNF and the GM-CSF levels in macrophage-lymphocyte cultures and an increase of the release of GM-CSF in blood lymphocyte cultures [1]. The experiments included in this paper represent...
part of the effort to understand the functional implications of the thymomodulin-dependent cytokine release.

As a first step, since TNF has the ability to synergize with GM-CSF in the induction of HLA-DR molecule expression on blood monocytes and on mononuclear phagocytes [6, 8], we evaluated HLA-DR antigen expression by macrophages and observed an increased HLA-DR expression in cultures with thymomodulin. Consistent with the fact that increased levels of TNF and GM-CSF were observed only in macrophage-lymphocyte co-cultures with thymomodulin [1], the increased HLA-DR expression was present only when macrophages were co-cultured with autologous lymphocytes, and not in cultures of macrophages alone. These data suggest that one of the effects of the cytokines released in increased amounts in cultures with thymomodulin is the enhancement of HLA-DR expression by macrophages.

The presence of surface HLA-DR molecules is a necessary requirement for the ability to present antigens and to induce the proliferation of T-lymphocytes [2, 3, 9, 21, 22]. The enhanced macrophage Class II molecule expression induced by thymomodulin, however, was not associated with changes in T-cell proliferation in the autologous mixed leucocyte cultures between alveolar macrophages and blood lymphocytes.

In this context, it has been shown that the in vitro T-cell proliferation is not better stimulated by macrophages as compared with monocytes, despite the fact that a higher proportion of AMs than blood monocytes express HLA-DR surface antigens [2, 3, 9]. Therefore, it is recognised that higher HLA-DR expression does not necessarily mean better antigen presenting function, at least as judged by these in vitro assays. In other systems, such as co-cultures of HLA-DR positive bronchial epithelial cells and of autologous T-cells, the expression of HLA-DR molecules, although sustained by γ-IFN, is not coupled with the functional capability to stimulate autologous T-cell proliferation in MLR [22]. This evidence suggests that these in vitro assays might not be sensitive enough or that the increased levels of HLA-DR expression on the surface of a given cell population may be relevant in other types of cellular interactions.

Thymic biological response modifiers are believed to act primarily on lymphocyte. Consistently with this concept, the effects of thymomodulin on other cell types, such as on macrophage cytokine release [1] and HLA-DR expression, are probably mediated through the changes induced by the compound on T-cells. The increased proliferation of T-lymphocytes stimulated with different mitogens induced by thymomodulin is consistent with the known effects of thymic hormones and peptides, including thymomodulin [1, 10-14, 23].

Although the presence of a primary or metastatic carcinoma of the lung may be associated with abnormalities of cell functions of immune related cells, in this study and in other experiments with human ciliated bronchial epithelial cells [22], we did not find any obvious difference between cancer and non-cancer patients for HLA-DR expression, and T-cell proliferation. Nevertheless, the data from this paper do not rule out the possibility that the effects of thymomodulin on cells, including macrophages and lymphocytes, from patients with cancer, might be different from the effects on cells of patients without cancer.

Finally, although some of the in vitro effects of thymomodulin described in this paper and in a study on cytokine production [1] are observed at concentrations of the compound considerably higher than the expected in vivo tissue concentrations [16, 17], animal studies suggest that a tissue concentration similar to those that we used in the in vitro assays could be safely reached in humans, either with a systemic or a topical administration. This observation might suggest the possibility of increasing the amounts of thymomodulin administered in clinical practice, at least in selected patients.

Acknowledgements: This work was performed as part of the Ph.D. programme of B. Balbi in Respiratory Diseases supported by the Interuniversitary Center of Northern Italy for Lung Diseases and the Italian Ministry of University and Scientific and Technologic Research. The authors are grateful to F. Trave and A. Tiri, Elleen Sri, for their help in conducting this work.

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