

SUPERNATANTS OF HUMAN LEUKOCYTES CONTAIN MEDIATOR, DIFFERENT FROM INTERFERON γ , WHICH INDUCES EXPRESSION OF MHC CLASS II ANTIGENS

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The expression of MHC class II antigens is not a constitutive property of nonlymphoid cells, but is dependent on lymphokines, as has been reported for murine (1–3) and human monocytes (4), endothelial cells, both in vivo (5, 6) and in vitro (7, 8), fibroblasts (9), tumor cells (10, 11), and brain cells (12). This effect of lymphokines on MHC class II antigen expression is influenced by eicosanoids (13), corticosteroids (14), and by the immunosuppressive drug cyclosporine A (5, 6, 8). IFN- γ has been suggested (15) as the responsible factor in supernatants of activated lymphocytes for antigen expression. Purified cDNA-derived IFN- γ possesses the capacity to induce expression of MHC-class II antigens (4, 7, 9–11).

In our experiments, however, a discrepancy was observed between IFN- γ titers in supernatants and the MHC class II antigen-inducing capacity. This finding suggested that MHC class II antigen expression is not only regulated by IFN- γ , but also by a combination of incompletely defined mediators in leukocyte supernatants.

In our search for the regulatory mechanism in MHC class II antigen expression, we studied immunoregulatory factors different from IFN- γ in supernatants of human leukocytes with respect to their MHC class II antigen-inducing capacity. Here we describe a mediator, different from IFN- γ , that induces MHC class II antigen expression. This mediator was present in supernatants of human leukocytes, and its effect appeared to be potentiated by IFN- γ . Biochemical analysis revealed a molecular weight of 32 kD.

Materials and Methods

Cells. Human diploid fibroblasts isolated from fetal lung tissue were kindly provided by Dr. Galama, Dept. of Virology, Hospital St. Annadal, Maastricht, The Netherlands. Human large bowel adenocarcinoma cells COLO205 and Hs703T were gifts of Dr. Verstijnen, Dept. of Pathology, University of Limburg. Kidney epithelial cells were isolated from human kidneys by trypsinization. Two kidneys from different donors have been used, harvested by our own center, and for medical reasons were rejected for

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transplantation use. Fibroblasts, kidney epithelial cells, and tumor cells were cultured in DMEM (Gibco, Paisley, United Kingdom) supplemented with 10% heat-inactivated (30 min, 56°C) FCS and antibiotics.

Reagents. IL-1 was purchased from Genzyme (Boston, MA) as purified natural human IL-1. Human cDNA-derived rIFN- γ and rIL-2 were kindly provided by Dr. Devos, Ghent, Belgium (16–18). Mixed leukocyte culture supernatants (MLC-SN)¹ were prepared as usual. Leukocytes were prepared by buoyant density centrifugation on Lymphoprep (Nyegaard, Oslo, Norway) of buffy coats of blood donors. Responder leukocytes, 10⁶ cells/ml, were cocultured with 4,000 rad x-irradiated stimulator leukocytes, 10⁶ cells/ml, in RPMI 1640 (Gibco) supplemented with 10% human serum and antibiotics. MLC-SN was harvested after 6 d of culture. For supernatant of lectin-stimulated leukocytes (PHA-SN), leukocytes 10⁶ cells/ml in medium identical to medium for MLC, were stimulated with PHA, used as purified PHA (Wellcome, Beckendham, United Kingdom) at 1 μ g/ml, for 3 d. Supernatants of MLC and PHA-stimulated leukocytes with a [³H]TdR uptake >25 times than that of control cultures were used throughout the experiments. MLC-SN, PHA-SN, or supernatants of unstimulated leukocytes were centrifuged, filter sterilized (pore size 0.22 μ m) and stored in aliquots at -20°C until use. Acid dialysis of supernatants was performed against a glycine-HCL buffer of pH 2 for 24 h, followed by dialysis against medium to obtain a neutral pH. MLC-SN and acid-dialyzed MLC-SN were concentrated 10-fold by dialysis against PEG 20,000 before IFN- γ titers were determined. IFN- γ titers in MLC-SN were determined in a virus protection assay with HEp-2 cells. In MLC-SN, IFN- γ titers ranged from 8 to 260 IU/ml. After acid dialysis, virus protection could not be detected; titers of IFN- γ were therefore lower than 0.2 IU/ml. For purpose of IFN- γ neutralization by mAb D9D10 (specific for human IFN- γ) twice the amount of anti-IFN- γ antibodies necessary for neutralization (as assayed in a virus-protection assay) was used for the supernatants. For the experiment with 100 IU rIFN- γ , the amount of antibody necessary to neutralize 200 IU was used. Addition of an irrelevant control mAb, F3-20-7 (against canine Thy-1 antigens [19]), in an identical dilution of ascites as used for mAb D9D10 had an effect similar to untreated supernatants (data not shown). The hybridoma secreting mAb D9D10 (IgG1) was derived through standard procedures. BALB/c mice were immunized with highly purified natural human IFN- γ . Screening of the fusion products was based on binding of natural human IFN- γ . The antibody D9D10 binds both natural and recombinant IFN- γ . Furthermore, it inhibits the effect of both types of human IFN- γ in a virus-protection assay.

Induction and Detection of MHC Antigens. Cells were incubated for 3 d in culture medium supplemented with reagents to be tested for capacity to induce expression of MHC antigens. Expression of MHC antigens was studied with an indirect immunofluorescence technique. The first layer consisted of mAbs against human MHC antigens; a second layer of FITC-labeled antiserum against mouse Ig was used (Nordic, Tilburg, The Netherlands). The mAbs Tü22, Tü36, and Tü39 (20) (reacting respectively against DQ, DR, and DR + DQ) were kindly provided by Dr. Ziegler, Medizinische Universitätsklinik, Tübingen, Federal Republic of Germany. The mAb Genox 3.53 (21), reacting with DQ, Medizinische Universitätsklinik, was a gift of Dr. W. Bodmer, Imperial Cancer Research Fund, London. In addition, mAb 7.5.10.1 (22), broadly reacting with MHC class II antigens, was used. The mAb W6/32 (23), reacting with MHC class I antigens, was purchased from Serotec (Blackthorn, United Kingdom). mAb F3.20.7 (19) served as control antibody in the immunofluorescence assay. Cells were stained in suspension in the presence of 0.1% BSA and 0.01% azide on ice. Both layers were incubated for 30 min, followed by three washes at 4°C. Fluorescence was determined with either a fluorescence microscope or with a FACS IV, and cells stained above control were considered positive for expression of MHC antigens.

Biochemical Analysis. Two antisera were immobilized on CNBr-activated Sepharose 4 B (Pharmacia Fine Chemicals, Uppsala, Sweden): ascites fluid from clone D9D10 (mouse anti-human IFN- γ), and the IgG fraction of a goat antiserum directed against semipurified

¹ Abbreviations used in this paper: FPLC, fast protein liquid chromatography; MLC, mixed leukocyte culture; SN, supernatant.

human IFN- γ (24). 100 ml of supernatant from a 6-d MLC was passed over the D9D10 Sepharose 4 B column (0.9 \times 12 cm, 10 ml/h, 4°C). The flow-through fractions, which did not contain any detectable human IFN- γ activity as titrated on HEp-2 cells, were then passed over the polyclonal goat anti-semipurified human IFN- γ column (0.9 \times 12 cm, 10 ml/h, 4°C). After washing the column with 20 mM Tris HCl, 0.15 M NaCl, pH 8.0, the bound substances were eluted with 0.1 M citrate, 0.5 M NaCl, pH 2. After localization of the fractions containing activity of MHC class II expression induction, they were pooled and extensively dialyzed against 50 mM formiate buffer, pH 4, and applied to a mono-S column in a fast protein liquid chromatography (FPLC) system (Pharmacia Fine Chemicals). No detectable activity was present in the flow-through or wash fractions, but upon application of a linear gradient of 0–0.65 M NaCl, the activity was eluted as a single peak. 2 ml of active fractions were concentrated by dialysis against PEG 20,000 to 300 μ l. To determine the molecular mass of the active mediator in these fractions, 100 μ l of this concentrated material was subjected to gel filtration on a TSK-G 3000 SW column (7.5 \times 600 mm; LKB-Produkter AB, Bromma, Sweden). This column had been calibrated with a mixture of BSA (68 kD), ovalbumin (43 kD), chymotrypsinogen (25 kD), and lysozyme (14.4 kD).

Results

MHC Class II Antigen-inducing Factor Different from IFN- γ . Supernatants from human leukocyte cultures were tested for their capacity to induce the expression of MHC class II antigens. For this study, four human cell types have been used. Human fibroblasts are known to express MHC class II antigens after incubation with rIFN- γ (9). Renal tubular epithelium is able to express the antigens in vivo in man (25–27). In addition to these cells, two human tumor cell lines, originating from large bowel adenocarcinomas and reported (10, 11) to express MHC class II antigens in vitro only after incubation with IFN- γ , were used as test cells to detect MHC class II antigen-inducing capacity.

MLC-SN induced MHC class II antigen expression, as shown in Table I. Since we observed a discrepancy between the MHC class II antigen-inducing capacity and the titer of IFN- γ (data not shown), inactivation of IFN- γ was introduced to study other putative factors involved. The effect of such supernatants could not be due solely to IFN- γ , since inactivation of IFN- γ did not abolish the effect. Both acid treatment (i.e., dialysis against a buffer of pH 2), and addition of mAbs against human IFN- γ did not prevent the induction of MHC class II antigens on fibroblasts, epithelial cells, and tumor cells. Inactivation of rIFN- γ by adding mAb abrogated the induction of the antigens by this lymphokine.

The dose-response effect of the newly discovered mediator was studied by incubation of the test cells with serial dilutions of leukocyte supernatants. Supernatants were dialyzed against a buffer of pH 2 or supplemented with mAb against human IFN- γ to study solely the influence of this factor on the expression of MHC class II antigens. Untreated MLC-SN could be diluted 1:16 with unconditioned medium to result in 90% of the test cells expressing MHC class II antigens. Neutralization of IFN- γ by mAb reduced the effect, though at a dilution of 1:16 the effect could still clearly be detected. Acid dialysis of the supernatants similarly reduced the effect, but did not abolish induction of MHC class II antigen expression (Fig. 1).

In addition, we studied the effect of activation of leukocytes on the production of MHC class II antigen expression-inducing mediator. The supernatant of unstimulated leukocytes was titrated and compared to titration of activated

TABLE I
Induction of MHC Class II Antigen Expression

Supernatant	Treatment	IFN- γ titer (IU/ml)	Percent positive cells			
			Fibro- blasts	Kidney epithelial cells	COLO 205	Hs703T
MLC-SN I	None	126	50	25	90	80
	Anti-IFN- γ added	ND*	30	15	80	40
	Acid dialyzed (pH 2)	ND	20	15	30	20
MLC-SN II	None	250	65	52	85	60
	Anti-IFN- γ added	ND	50	36	50	60
	Acid dialyzed (pH 2)	ND	30	46	50	40
rIFN- γ	None	100	40	35	99	95
	Anti-IFN- γ added	ND	— [‡]	—	—	—
Culture medium	None	ND	—	—	—	—

The results of two different MLC-SN are shown, which are representative for a series of 16 supernatants tested extensively. As indicated in Materials and Methods, cells were incubated for 3 d with the reagents to be tested. MHC class II antigen expression was determined with mAb 7.5.10.1.

* Virus-protective effect of IFN- γ was not detectable.

[‡] Positive cells were not detected.

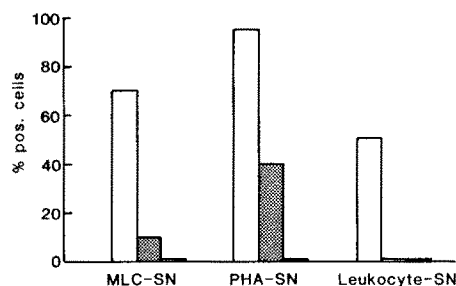


FIGURE 1. MHC class II induction by incubation of COLO205 cells for 3 d with dilutions (open bars, 1:4 dilution; stippled bars, 1:16; striped bars, 1:64) of acid-dialyzed supernatants of alloactivated, lectin-activated, and nonactivated leukocytes. Nonactivated leukocytes (10^6 cells/ml) were cultured for 6 d (as in MLC) in complete medium. MHC class II expression was determined with mAb 7.5.10.1.

leukocyte supernatants. As can be seen in Fig. 1, unstimulated leukocytes produced lower concentrations of the factor than stimulated leukocytes. The presence of the factor in supernatants of unstimulated leukocytes was, however, still clearly detectable at dilutions of 1:4. In lectin-stimulated cultures, we observed concentrations comparable to concentrations produced in MLC.

From these experiences, we conclude that a mediator, different from IFN- γ , with capacity to induce MHC class II antigen expression is present in the supernatants of human leukocytes.

Kinetics of Appearance of MHC Class II Antigens. The expression of MHC class II antigens of the test cell COLO205 incubated with the newly described mediator or with IFN- γ was studied with different incubation times. Results of

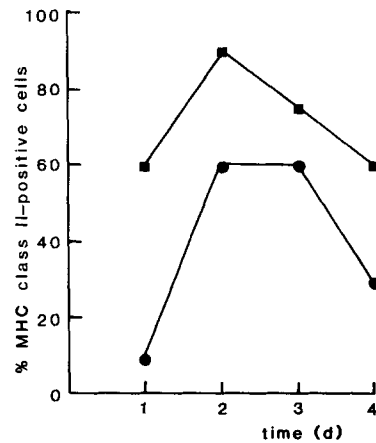


FIGURE 2. Kinetic response of MHC class II antigen expression induction by IFN- γ (■) and by mediator from leukocyte supernatant (●). COLO205 was used as test cell, and antibody 7.5.10.1 was used to detect antigen expression. IFN- γ was used in a concentration of 2 IU/ml; mediator from leukocyte supernatant was used as acid-dialyzed MLC-SN at a concentration of 75%. At initiation of the experiments MHC class II antigens could not be detected on COLO205.

a representative experiment are presented in Fig. 2. It appeared that both the mediator from MLC-SN and IFN- γ induced antigen expression that could be detected as early as day 1 of incubation. The effect of IFN- γ reached its maximum at day 2 and declined afterwards. The effect of the mediator in MLC-SN increased till day 2, remained at a constant level until day 3, and declined thereafter. Lower concentrations of either IFN- γ or the mediator had reduced effects on antigen expression with similar kinetics (data not shown).

Comparison with Known Factors. In addition to acid dialyzed leukocyte SN, a number of well-defined factors have been studied for capacity to induce MHC antigens. MHC class I antigens and subsets of MHC class II antigens were studied. Purified IL-1 induced a marked increase in expression of MHC class I antigens (Table II). An effect of IL-1 on MHC class II antigen expression could not be detected. IL-2 affected neither MHC class I nor MHC class II antigen expression. IFN- γ increased both MHC class I and MHC class II expression, as reported (28, 29). The effect of IFN- γ on the expression of the molecules of the MHC class II gene complex differed for the subsets; HLA-DR was clearly increased, whereas only a weak effect on HLA-DQ was observed, as similarly reported by Collins et al. (29). Acid-treated MLC-SN induced a strong increase in MHC class I antigens and HLA-DR gene products, and a significant increase of HLA-DQ gene products. Results are presented in Table II.

Interaction. The MHC class II antigen-inducing capacity of leukocyte supernatants observed in the above-described experiment appeared to be reduced after neutralization of IFN- γ . This observation suggested that IFN- γ present in untreated supernatants potentiated the MHC class II antigen-inducing capacity of the mediator under study. In analysis of this putative interaction, low concentrations of IFN- γ were added to titrations of acid-dialyzed MLC-SN. It appeared that IFN- γ , at concentrations with submaximal effects on induction of MHC

TABLE II
Effect of Defined Cytokines on Expression of MHC Antigens

MHC structure	mAb	Percent positive cells after incubation with:				
		IL-1 (6 U/ml)	rIL-2 (100 ng/ml)	rIFN- γ (50 IU/ml)	MLC-SN + anti- IFN- γ	Medium
Class I	W6/32	99+++	95+	99++++	99++++	95+
Class II broad	7.5.10.1	—	—	99	95	—
Class II DR + DQ	Tü39	—	—	95	95	—
Class II DR	Tü36	—	—	30	70	—
Class II DQ	Tü22	—	—	5	60	—
Class II DQ	Genox 3.53	—	—	5	50	—

The percentage of positive cells of tumor COLO205, determined after a 3-d incubation period, is indicated. A representative experiment from a series of four experiments is given. Intensity of staining with anti-MHC class I marker was arbitrarily expressed and ranged from normal to very strong, (+ to ++++ respectively).

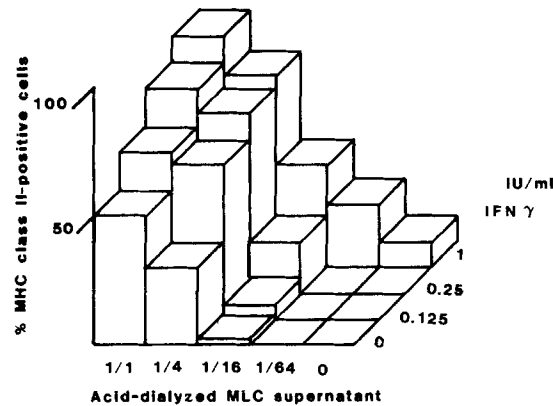


FIGURE 3. Interaction of IFN- γ and MHC class II antigen-inducing factor. COLO205 cells were incubated for 3 d with dilutions of acid dialyzed MLC-SN, to which various concentrations of IFN- γ were added. Percentage of MHC class II-positive cells was determined with mAb 7.5.10.1.

class II antigen, markedly increased the expression of MHC class II antigens induced by the mediator, as shown in Fig. 3. In the opposite experiment, using a fixed dilution of mediator-containing acid-dialyzed MLC-SN and a titration of IFN- γ , we observed a similar potentiating effect of IFN- γ on induction of MHC class II antigens by the mediator. The effect of the combined reagents exceeded the effects of the summation of the separate reagents. The potentiating effect of these two reagents explains the reduction in effect on induction of MHC class II antigens after neutralization of IFN- γ in leukocyte supernatants: after neutralization, only the effect of the mediator under study was observed, unaffected by the potentiating effect of IFN- γ .

Biochemical Analysis. The unidentified mediator was biochemically characterized. At first we studied whether MHC class II antigens present in the leukocyte supernatants could explain the presence of MHC class II antigens on the test cells after incubation with leukocyte supernatants. The mAb 7.5.10.1, which

TABLE III
Binding of Mediator to Different Antibody Columns

Supernatant	Treatment	Percent positive cells*	Intensity
MLC-SN	—	95	4
SN I	After passage over D9D10 [‡]	95	3
SN II	After passage over 1395 [‡]	20	1
SN III	Elution from 1395 [§]	95	3

* COLO205 was used as test cell, and mAb 7.5.10.1 was used as first layer for detection of MHC class II antigens. Response is shown as percent positive cells. Intensity of fluorescence is arbitrarily defined as: 4, very strong; 3, strong; 2, clearly detectable; and 1, respectively weak.

[‡] Antibody D9D10 is a mAb against human IFN- γ ; 1395 is a goat antiserum against semipurified human IFN- γ (24).

[§] Response of the eluate after dialysis and dilution to the volume originally applied to column D9D10.

appeared to be highly efficient in removing MHC class II antigens from cell extracts (30), was bound to Sepharose beads and used for extensive immunoabsorption of acid-dialyzed MLC-SN. Subsequently, treated and untreated MLC-SN were titrated in serial dilutions. Immunoabsorption did not remove the inducing capacity from these acid-dialyzed supernatants as shown by an experiment in which, before absorption, 80% of the cells were positive for induced antigen expression, whereas after absorption, 75% of the cells were MHC class II-positive. We therefore conclude that the mediator must be antigenically different from MHC class II antigens.

A next step in the identification of the mediator was based on the observation that the activity of the supernatants could not be neutralized by mAb against IFN- γ , but was neutralized by a polyclonal goat antiserum prepared against semipurified IFN- γ derived from activated human leukocytes (24). In a first attempt to isolate and purify the mediator, the crude supernatants from 6-d MLC were passed consecutively over two columns of antibodies immobilized on Sepharose 4 B: monoclonal mouse anti-human IFN- γ on the first one, and polyclonal goat anti-semipurified human IFN- γ on the second. Table III shows the results of one representative experiment out of a series of six. The second column was eluted with pH 2 buffer, and after localization of the biological activity, fractions were pooled, dialyzed against formate buffer, pH 4, and applied to a mono-S column in an FPLC system. Upon application of a linear NaCl gradient (0–0.65 M NaCl) to such column, the factor eluted as a single peak of activity of antigen expression induction at a salt concentration of 0.45 M NaCl (Fig. 4a). This peak fraction was then concentrated and subjected to high-performance gel filtration on a TSK-G 3000 SW column. A single peak of activity was obtained, which eluted at a point corresponding to a molecular mass of 32 kD (Fig. 4b). The results of biochemical analysis show that: (a) the activity is not neutralized by acid treatment, (b) the activity is not neutralized by mAb against human IFN- γ , (c) the activity is antigenically different from MHC class II antigens, (d) the activity was neutralized by polyclonal goat antiserum against semipurified human IFN- γ , (e) the activity binds to cation-exchange column on FPLC, (f) the activity has a molecular mass of 32 kD by FPLC gel filtration.

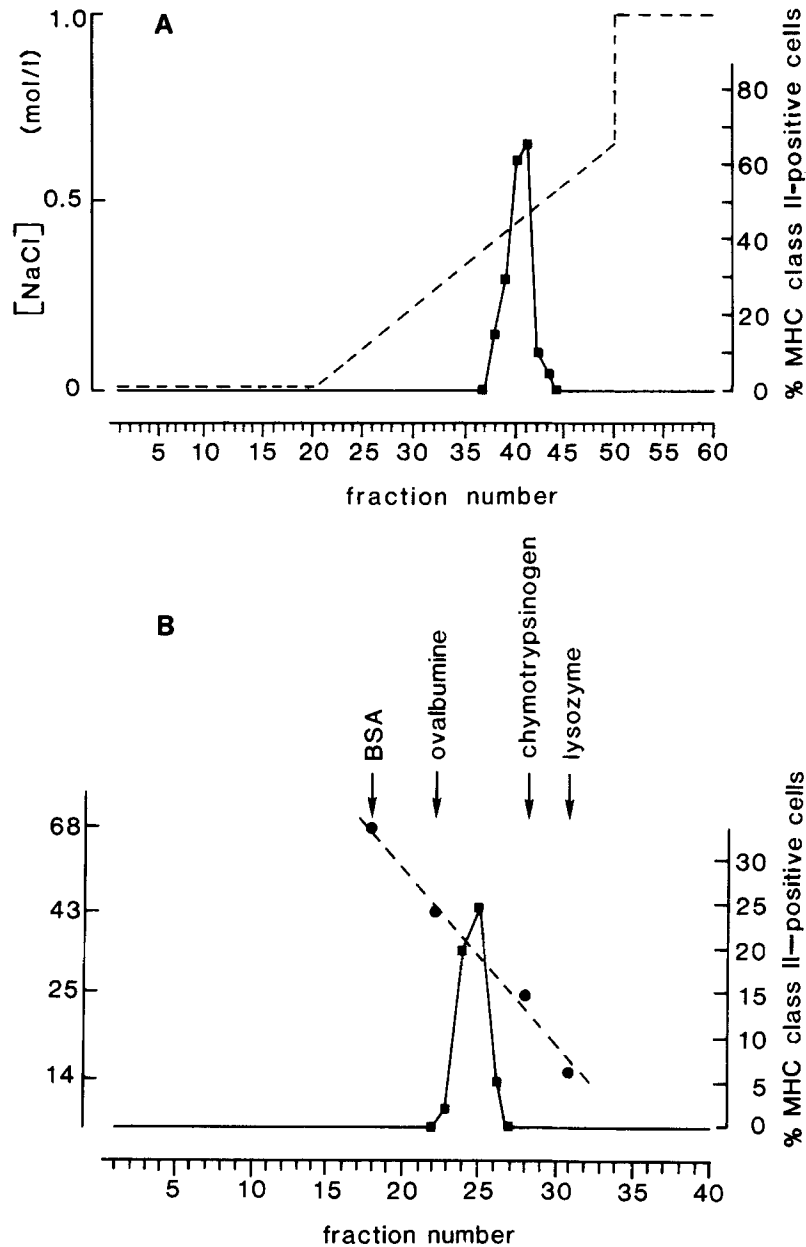


FIGURE 4. A, Profile on FPLC mono-S column of mediator from MLC-SN. Eluate of antibody column 1395, dialyzed against sample buffer and concentrated, was applied to the column. The MHC class II antigen expression-inducing capacity was eluted using a 0–0.65 M NaCl gradient. Activity eluted as a single peak, highest activity at 0.45 M NaCl, and the responses of the fractions forming this peak have been marked. All other fractions showed a negative reaction. The four fractions with highest activity were pooled and used for further purification. B, Profile on FPLC TSK-C3000 SW column of mediator from MLC-SN. To determine molecular mass (ordinate, in kD) of the mediator, protein standards were run in parallel, as indicated. Pooled fractions from mono-S column (2 ml) were concentrated to 0.3 ml, and 0.1 ml of this solution was applied to the column. A single peak of activity, corresponding to a molecular mass of 32 kD, was observed. Responses of the fractions forming this peak have been marked. All other fractions showed a negative reaction.

Discussion

In this report, we describe a mediator, different from IFN- γ , which induces expression of MHC-class II antigens. The mediator is present in supernatants of human leukocytes and affects antigen expression on a number of different human cell types, such as fibroblasts, epithelial cells, and tumor cells. In the mouse (32, 33) suggestive evidence has been produced for a non-IFN- γ mediator with a similar effect on MHC class II antigen expression. A soluble product of murine macrophages increases expression of Ia-antigens IFN- γ (31), and an IFN- γ -induced tumor macrophage factor with Ia-inductive capacity (32) has been reported. In addition to these murine mediators, a human mediator in leukocyte supernatants, different from IFN- γ , has now been identified.

The difference between the mediator, reported here, with capacity to induce MHC class II antigen expression, and human IFN- γ can be concluded from the findings discussed below. The two substances are antigenically and physicochemically different. The MHC class II antigen-inducing mediator is resistant to acid treatment, whereas IFN- γ is not. The mediator binds to a cation-exchange column, while IFN- γ does not bind to such a column under the conditions used. Also the molecular masses are different, 32 kD for the mediator vs. 45 kD for IFN- γ . mAbs generated against purified human IFN- γ , which when immobilized bind and remove, and when in solution neutralize IFN- γ , neither remove the mediator nor affect its activity. Furthermore, the kinetic response of induction of antigen expression by the mediator from MLC-SN makes it unlikely that this antigen expression is caused by MHC class II molecules adhering to the test cells. In addition, absorption with antibody against MHC class II antigens did not remove the activity of the mediator from the MLC-SN, excluding the possibility that the mediator is, itself, of that class.

It is unlikely that the mediator is related to the IFN- γ molecule. The mediator does not share a physicochemical or antigenic characteristic of IFN- γ , as discussed above. In addition, prolonged acid treatment of IFN- γ , resulting in inactivation of this lymphokine, did not generate a capacity to induce expression of MHC class II antigens that could not be inhibited by mAbs against human IFN- γ . Therefore, the mediator described in this report is not produced during acid inactivation of IFN- γ . Moreover, addition of mAbs against IFN- γ to non-acid-dialyzed MLC-SN showed that the mediator is also present in these supernatants.

Functionally, IFN- γ and the new mediator are dissimilar. IFN- γ has a potent virus-protective effect, which the mediator obviously lacked. IFN- γ results in only a minor increase of HLA-DQ antigens (29), whereas the effect of the mediator on HLA-DQ antigen expression is much more pronounced, at concentrations of these two substances that result in comparable effects on increased reactivity with a broadly reactive anti-MHC class II mAb. Subsequently, it is noteworthy that the combined action of the two factors results in potentiation of the effect.

The description of this mediator, different from IFN- γ , with capacity to induce expression of MHC class II antigens, sheds new light on the regulation of antigen expression. The interaction observed between the mediator and IFN- γ might be explained by enhanced induction (by either mediator) of receptors for itself or the other mediator, which would allow fine regulation of antigen expression

during immunological processes. It should, however, be mentioned that the new mediator is capable of inducing antigen expression in the absence of even a trace amount of IFN- γ . It could therefore be imagined that IFN- γ induces cells to produce the new mediator, which itself eventually induces MHC class II antigen expression (27). In such an interactive system, many points of actions for modulators of MHC class II antigen expression, such as prostaglandins (13) and corticosteroids (14) can be envisaged.

It can be hypothesized that the described mediator is involved not only in vitro, but also in vivo in immunological reactions via regulation of MHC class II antigen expression, and might represent a new interleukin. Study of this factor in hyperimmune situations such as allograft rejection and autoimmune disease, and in hypimmune situations such as the bare lymphocyte syndrome and the acquired immunodeficiency syndrome, all associated with changes in MHC class II antigen expression (respectively increased [25, 33–36] and decreased [37, 38]), might offer new insights into these situations and into the involvement of this mediator in regulation of MHC class II antigen expression.

Summary

In this report, data are presented on the regulation of MHC class II antigen expression by a mediator present in supernatants of human mixed leukocyte cultures (MLC-SN), and which is different from IFN- γ . The capacity of supernatants to induce antigen expression did not correspond to titers of IFN- γ . Removal of IFN- γ using either dialysis against pH 2 or neutralizing mAb against human IFN- γ did not abrogate the MHC class II antigen expression-inducing capacity of MLC-SN when tested on adenocarcinoma cell lines, kidney epithelial cells, and fibroblasts in vitro in an indirect immunofluorescence assay. Therefore, supernatants of human leukocytes contain a mediator, different from IFN- γ , which induces expression of MHC class II antigens. Dose-response studies revealed that the mediator is produced after allogeneic and lectin stimulation of human leukocytes, and by unstimulated leukocytes. Activation of leukocytes resulted in increased titers of the mediator. The mediator markedly enhances expression of both HLA-DR and HLA-DQ antigens, whereas IFN- γ had a similar effect on HLA-DR antigens, and only a minor effect on HLA-DQ antigens. Interaction of the mediator and IFN- γ resulted in a potentiating effect of these two factors on MHC class II antigen expression. Biochemical analysis revealed a mediator, distinguishable by FPLC from IL-1, IL-2, and human IFN- γ , and which has a molecular mass of 32 kD.

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References

1. Steinman, R. M., N. Noguera, M. D. Witmer, J. D. Tydings, and J. S. Mellman. 1980. Lymphokine enhances the expression and synthesis of Ia antigens on cultured mouse peritoneal macrophages. *J. Exp. Med.* 152:1248.
2. Steeg, P. S., R. N. Moore, and J. J. Oppenheim. 1980. Regulation of murine

- macrophage Ia-antigen expression by products of activated spleen cells. *J. Exp. Med.* 152:1734.
3. Scher, M. G., D. J. Beller, and E. R. Unanue. 1980. Demonstration of a soluble mediator that induces exudates rich in Ia-positive macrophages. *J. Exp. Med.* 152:1684.
 4. Szein, M. B., P. S. Steeg, H. M. Johnson, and J. J. Oppenheim. 1984. Regulation of human peripheral blood monocyte DR antigen expression in vitro by lymphokines and recombinant interferons. *J. Clin. Invest.* 73:556.
 5. de Waal, R. M. W., M. J. J. Bogman, C. N. Maass, L. M. H. Cornellissen, W. J. M. Tax, and R. A. P. Koene. 1983. Variable expression of Ia-antigens on the vascular endothelium of mouse skin allografts. *Nature (Lond.)* 303:426.
 6. Groenewegen, G., W. A. Buurman, and C. J. van der Linden. 1985. Lymphokine dependence of in vivo expression of MHC class II antigens by endothelium. *Nature (Lond.)* 316:361.
 7. Pober, J. S., M. A., Gimbrone, R. S. Cotran, C. S. Reiss, S. J. Burakoff, W. A. Fiers, and K. A. Ault. 1983. Ia expression by vascular endothelium is inducible by activated T cells and by human gamma-interferon. *J. Exp. Med.* 157:1339.
 8. Groenewegen, G., W. A. Buurman, G. M. A. A. Jeunhomme, and C. J. van der Linden. 1985. Effect of cyclosporine on MHC class II antigen expression on arterial and venous endothelium in vitro. *Transplantation (Baltimore)* 40:21.
 9. Pober, J. S., T. Collins, M. A. Gimbrone, R. S. Cotran, J. D. Gitlin, W. Fiers, C. Clayberger, A. M. Krensky, S. J. Burakoff, and C. S. Reiss. 1983. Lymphocytes recognize human vascular endothelial and dermal fibroblasts. Ia antigens induced by recombinant immune interferon. *Nature (Lond.)* 305:726.
 10. Basham, T. Y., and T. C. Merigan. 1983. Recombinant interferon- γ increases HLA-DR synthesis and expression. *J. Immunol.* 130:1492.
 11. Pfizenmaier, K., H. Bartsch, P. Scheurich, B. Seliger, U. Ücer, K. Vehmeyer, and G. A. Nagel. 1985. Differential γ -interferon response of human colon carcinoma cells: inhibition of proliferation and modulation of immunogenicity as independent effects of γ -interferon on tumor cell growth. *Cancer Res.* 45:3503.
 12. Wong, G. H. W., P. F. Bartlett, I. Clark-Lewis, F. Battye, and J. W. Schrader. 1984. Inducible expression of H-2 and Ia antigens on brain cells. *Nature (Lond.)* 310:688.
 13. Snyder, D. S., D. J. Beller, and E. R. Unanue. 1981. Prostaglandins modulate macrophage Ia expression. *Nature (Lond.)* 299:163.
 14. Snyder, D. S., and E. R. Unanue. 1982. Corticosteroids inhibit murine macrophage Ia expression and interleukin production. *J. Immunol.* 129:1803.
 15. Steeg, P. S., R. N. Moore, H. M. Johnson, and J. J. Oppenheim. 1982. Regulation of murine macrophage Ia antigen expression by a lymphokine with immune interferon activity. *J. Exp. Med.* 156:1780.
 16. Scahill, S. J., R. Devos, J. van der Heyden, and W. Fiers. 1983. Expression and characterization of the product of a human immune interferon cDNA gene in Chinese hamster ovary cells. *Proc. Natl. Acad. Sci. USA.* 80:4654.
 17. Devos, R., H. Cheroutre, Y. Taya, W. Degrave, H. Van Heverswijn, and W. Fiers. 1982. Molecular cloning of human immune interferon cDNA and its expression in eukaryotic cells. *Nucleic Acids Res.* 10:2487.
 18. Devos, R., G. Plaetinck, H. Cheroutre, G. Simons, W. Degrave, J. Taverner, E. Remaut, and W. Fiers. 1983. Molecular cloning of human Interleukin 2 cDNA and its expression in *E. coli*. *Nucleic Acids Res.* 11:4307.
 19. McKenzie, J. L., and J. W. Fabre. 1981. Studies with a monoclonal antibody on the distribution of Thy-1 in the lymphoid and extracellular connective tissue of the dog. *Transplantation (Baltimore)* 31:275.

20. Pawelec, G., A. Ziegler, and P. Wernet. 1985. Dissection of human allostimulatory determinants with cloned T cells: stimulation inhibition by monoclonal antibodies Tü 22, 34, 36, 37, 39, 43 and 58 against distinct human MHC class II molecules. *Hum. Immunol.* 12:165.
21. Brodsky, F., P. Parham, and W. F. Bodmer. 1980. Monoclonal antibodies to HLA-DRw determinants. *Tissue Antigens.* 16:30.
22. Koning, F., J. Schreuder, M. Giphart, and H. Bruning. 1984. A mouse monoclonal antibody detecting a DR-related MT-2 like specificity. Serology and biochemistry. *Hum Immunol.* 9:221.
23. Barnstable, C. J., W. F. Bodmer, G. Brown, G. Galfre, C. Milstein, A. F. Williams, and A. Ziegler. 1978. Production of monoclonal antibodies to group A erythrocytes, HLA and other human cell surface antigens: new tools for genetic analysis. *Cell.* 14:9.
24. De Ley, M., J. Van Damme, A. Billiau, and P. De Somer. 1981. The preparation of antibodies directed against human immune interferon. *J. Virol. Methods.* 3:149.
25. Hall, B. M., G. A. Bishop, G. G. Duggin, J. S. Horvath, J. Philips, and D. J. Tiller. 1984. Increased expression of HLA-DR antigens on renal tubular cells in renal transplants: Relevance to the rejection response. *Lancet.* ii:247.
26. Hart, D. N. J., S. V. Fuggle, K. A. Williams, J. W. Fabre, A. Ting, and P. J. Morris. 1981. Localization of HLA-ABC and DR antigens in human kidney. *Transplantation (Baltimore).* 31:428.
27. Fuggle, S. V., P. Errasti, A. S. Daar, J. W. Fabre, A. Ting, and P. J. Morris. 1983. Localization of major histocompatibility complex (HLA-ABC and DR) antigens in 46 kidneys. *Transplantation (Baltimore).* 35:385.
28. Wallach, D., M. Fellous, and M. Revel. 1982. Preferential effect of γ -interferon on the synthesis of HLA antigens and their mRNAs in human cells. *Nature (Lond.).* 299:833.
29. Collins, T., A. J. Korman, C. T. Wake, J. M. Bass, D. J. Kappes, W. Fiers, K. A. Ault, M. A. Gimbrone, J. L. Strominger, and J. S. Pober. 1984. Immune interferon activates multiple class II major histocompatibility complex genes and the associated invariant chain gene in human endothelial cells and dermal fibroblasts. *Proc. Natl. Acad. Sci. USA.* 81:4917.
30. Doveren, R. F. C., C. J. van der Linden, E. E. M. Spronken, G. Groenewegen, and W. A. Buurman. 1986. Canine MHC-class II antigens on B and T lymphocytes. *Tissue Antigens.* 27:87.
31. Lonai, P., and L. Steinman. 1977. Physiological regulation of antigen binding to T cells: role of a soluble macrophage factor and of interferon. *Proc. Natl. Acad. Sci. USA.* 74:5662.
32. Walker, E. B., V. Maimo, M. Sanchez-Lanier, N. Warner, and C. Stewart. 1984. Murine gamma-interferon activates the release of a macrophage-derived Ia-inducing factor that transfers Ia inductive capacity. *J. Exp. Med.* 159:1532.
33. Lampert, I. A., A. J. Suitters, and P. M. Chisholm. 1981. Expression of Ia antigens on epidermal keratinocytes in graft-versus-host disease. *Nature (Lond.).* 293:149.
34. Mason, D. W., M. Dallman, and A. N. Barclay. 1981. Graft-versus-host disease induces expression of Ia antigens in rat epidermal cells and gut epithelium. *Nature (Lond.).* 293:150.
35. Ballardini, G., R. Mirakian, F. B. Bianchi, E. Pisi, D. Doniach, and G. F. Bottazzo. 1984. Aberrant expression of HLA-DR antigens on bileduct epithelium in primary biliary cirrhosis: relevance to pathogenesis. *Lancet.* iii:1009.
36. Hanafusa, T., R. Pujol-Borrell, R. Chiovato, R. C. G. Russel, D. Doniach, and G. F.

- Bottazzo. 1983. Aberrant expression of HLA-DR antigen on thyrocytes in Grave's disease: relevance for autoimmunity. *Lancet*. iii:1111.
37. Belsito, D. V., M. R. Sanchez, R. L. Baer, F. Valentine, and G. J. Thorbecke. 1984. Reduced Langerhans cell Ia antigen and ATPase activity in patients with the acquired immunodeficiency syndrome. *New Engl. J. Med.* 310:1279.
38. Griscelli, C., A. Fischer, B. GrosPierre, A. Durandy, C. Bremard, D. Charron, E. Vilmer, and J-L. Virelizier. 1984. Clinical and immunological aspects of combined immunodeficiency with defective expression of HLA-antigens. *In Progress in Immunodeficiency Research and Therapy*. I. C. Griscelli and J. Vossen, editors. Elsevier Scientific Publishing Co. 19-26.