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Immunology

Interferon-γ-like molecule induces Ia antigens on cultured mast cell progenitors

(P cells/lymphokines/antigen-specific T-cell clone/major histocompatibility antigens)

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ABSTRACT **Persisting (P) cells (murine cells that resemble mast cells and grow continuously *in vitro* for prolonged periods in the presence of a specific growth factor) did not express detectable levels of Ia antigens (murine class II major histocompatibility antigens) when their growth was supported by partially purified P cell-stimulating factor. However, when these Ia-negative P cells were transferred to medium conditioned by concanavalin A-stimulated spleen cells, Ia antigens appeared within 24 hr. The increase in Ia antigens was due to induction of synthesis of Ia antigens by P cells and not to absorption of Ia antigens from the conditioned medium or selective growth of Ia-positive cells from a low number of Ia-positive cells in the original population. The Ia-inducing activity was also found in supernatants from antigen-stimulated cloned T-cell lines, but not from certain T-cell hybridomas or the T lymphoma EL-4. The presence of Ia-inducing activity correlated with the presence of interferon-γ (IFN-γ). The gel filtration profiles of IFN-γ activity and Ia-inducing activity were coincident and corresponded to an apparent molecular weight of 40,000–45,000. Both the IFN-γ and Ia-inducing activity were destroyed by treatment at pH 2. These results indicate that IFN-γ or a closely related molecule induces Ia antigens on P cells and suggest that regulation of Ia antigen expression may be an important aspect of the effects of IFN-γ on the immune and hemopoietic systems.**

It is often assumed that the class II major histocompatibility antigens (1), the *I*-region-associated Ia antigens in mice and the HLA-D antigens in the human, function by presenting foreign antigens to T cells and are limited in their distribution to lymphocytes and cells specialized for antigen presentation (2). Although class II antigens have been reported on normal hemopoietic progenitor cells (3–5), Ia antigens can be transferred from one cell type to another (6), and the possibility remained that the class II antigens were not synthesized by hemopoietic progenitor cells but were passively absorbed.

Recently, we described a novel type of murine cell that had many structural and biochemical similarities to mast cells and grew continuously in culture. We gave these cells the operational name of persisting (P) cells because of their persistent growth. The characteristics of P cells suggest that they probably correspond to the progenitors of a subset of mast cells (7–9). The *in vitro* growth of P cells was dependent upon a T cell-derived factor, P cell-stimulating factor (PSF) (7–10), distinct from T-cell growth factor (TCGF) or T cell-derived granulocyte/macrophage colony-stimulating factor (GM-CSF) (10). We have found that P cells that had been cultured in the presence of medium conditioned by concanavalin A (Con A)-stimulated spleen cells (CAS medium) expressed high levels of Ia antigens on their cell surface (9). However, the *in vitro* expression of Ia antigens on cloned, homogeneous populations of rapidly dividing P cells clearly demonstrated that Ia antigens could be synthesized by cells distinct from lymphocytes or the recognized types of immunological accessory cells (9). Here we extend these observations and report that the expression of Ia antigens on these murine mast cell progenitors is under the control of a T cell-derived factor distinct from the factor (PSF) required for growth of these cells (10) but indistinguishable from interferon-γ (IFN-γ).

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MATERIALS AND METHODS

Preparation of Conditioned Medium. CAS medium was prepared by using spleens from CBA mice (*I-A*^{k}, *I-E*^{k}), (C57BL/6 × DBA/2) F_1 hybrid mice designated BDF_1 (*I-A*^{b}, *I-A*^{d}, *I-E*^{d}) or BALB/c mice (*I-A*^{d}, I-E^d). Media conditioned by Con A-stimulated T-cell hybridomas 123, T6, and T19.1 were prepared as described (11). Medium conditioned by the lymphoma EL-4 was prepared by culturing EL-4 cells at 2×10^6 cells per ml with 10% fetal calf serum for 24 hr in the presence of 12-*O*-tetradecanoylphorbol 13-acetate (10 ng/ml), followed by concentration of the supernatant 10-fold by using an Amicon hollow-fiber system. W3-C medium was a 10-fold concentrated supernatant of cultures in which the myelomonocytic tumor cells WEHI-3B had grown to $\approx 1.5 \times 10^6$ cells per ml. TCGF and PSF were partially purified from CAS medium by ammonium sulfate precipitation and hydrophobic chromatography (12).

Preparation of Supernatants from Cloned T-Cell Lines. Supernatants were obtained from antigenically stimulated T-cell clones derived from either a BALB/c anti-oxazalone line or an A/J anti-azobenzene arsonate line (13). Supernatants from clones cultured with normal lymph node cells were used as unstimulated controls.

Cell Culture. P cells were derived from CBA murine bone marrow cells cultured for 2 wk in medium (RPMI 1640) supplemented with 50 μM 2-mercaptoethanol, 100 μM L-asparagine, 2.8 mM glutamine, 10% fetal calf serum, and 3% CAS medium 10 times concentrated. P cells then were passaged biweekly in the same medium containing 3% W3-C medium instead of CAS medium. After 6 wk in cultures supplemented with W3-C medium, P cells grew as a homogeneous population of granulated cells that expressed barely detectable or undetectable levels of Ia antigens. For experiments, Ia-negative P cells ($\approx 5 \times 10^4$ cells per ml per well) were cultured in 24-well cul-

Abbreviations: CAS medium, medium conditioned by concanavalin A-stimulated spleen cells; Con A, concanavalin A; IFN-γ, interferon-γ; P cell, persisting cell; PSF, P cell-stimulating factor; PRD_{50}, 50% plaque-reducing dose; W3-C medium, medium conditioned by the myelomonocytic tumor WEHI-3B; MHC, major histocompatibility complex; FACS, fluorescence-activated cell sorter; TCGF, T-cell growth factor; GM-CSF, granulocyte/macrophage colony-stimulating factor.

ture trays (Linbro 76-033-05) at 37°C in humidified 10% CO_2/90% air. Although we have found that high concentrations of partially purified PSF or W3-C medium have an antagonistic effect on the induction of Ia antigens by CAS medium, this was not evident at lower concentrations that still maintained growth. Therefore, in all experiments testing the Ia-inducing activity of various supernatants or fractions thereof, low concentrations of W3-C medium (0.1%) were included to ensure that P cells remained viable.

Detection of Antigens on P Cells. Ia antigens were detected by indirect immunofluorescence with two monoclonal anti-Ia antibodies [10-2-16 binding to a determinant coded for by $I\text{-}A^k$ (14) or 14.4-4S binding to a determinant coded for by $I\text{-}E^k$ and $I\text{-}E^d$ (15)] and by fluorescein-conjugated sheep anti-mouse Ig. Staining was analyzed by counting at least 400 viable cells per duplicate sample under the fluorescence microscope or by using a fluorescence-activated cell sorter (FACS; a modified Becton and Dickinson FACS II) to determine the mean intensity of fluorescence per sample on a logarithmic scale (in arbitrary units), a minimum of 4,000 cells being analyzed per sample. All experiments were performed at least three times, and the results were reproducible.

Similar techniques were used to measure the levels of binding of monoclonal antibodies to antigens H-$2K^k$, 11.4-1 (14), and Ly 5 (obtained from I. McKenzie, University of Melbourne) and to an Fc fragment receptor (obtained from A. Lopez, The Walter and Eliza Hall Institute). Fluorescein-coupled peanut agglutinin (Sigma) was used to measure levels of receptors for peanut agglutinin.

Interferon Assay. Interferon was assayed by inhibition of plaque formation by Semliki Forest virus as described (13). Titers are expressed in laboratory units as the reciprocal of the dose causing a 50% reduction in the number of plaques (PRD_{50}) compared to the control plates. One laboratory unit is approximately equal to one unit of the World Health Organization's international reference preparation of α and β interferon no. G-002-904-511, supplied by the National Institute of Allergy and Infectious Diseases.

Incorporation of [^{3}H]Thymidine. Six 10-μl aliquots of P cells ($\approx 5 \times 10^3$ cells) were taken from each of the 1-ml cultures and were cultured for 24 hr in the wells of a Terasaki plate with 1 μCi (1 Ci = 3.7×10^{10} becquerels) of [^{3}H]thymidine. Trichloroacetic acid-precipitable materials were then harvested with a Titertek cell harvester designed for use with Terasaki plates.

Gel Filtration. Gel filtration on a Sephadex G-75 superfine column was carried out in phosphate-buffered saline exactly as described (10).

Acid Treatment. A 0.1 vol of 1 M glycine·HCl (pH 2.0) was added to the conditioned medium. Control supernatants were treated with 1 M Hepes (pH 7.0) in the same way. After 16 hr at 4°C, the media were dialyzed against 1,000 vol of phosphate-buffered saline.

RESULTS

Variable Expression of Ia Antigens on P Cells. When P cells from CBA mice were cultured with W3-C medium as a source of PSF, Ia antigens were undetectable on the majority of P cells, although there was a minority of cells (<1%) on which low levels of Ia could be detected by indirect immunofluorescence. When these P cells were transferred to medium supplemented with CAS medium, the levels of Ia antigens on their cell surface increased rapidly, with 50–70% and 80–100% of cells being unequivocally positive after 24 hr and 36 hr, respectively. P cells that were cultured in CAS medium were stained brightly with both monoclonal anti-Ia antibodies 10-2.16 and 14.4-4S, indicating that products of both the *I-A* and *I-E* loci were present. Control experiments indicated that, under the conditions used, the binding of these monoclonal antibodies was specific and not, for example, due to Fc receptors. Thus, the 10-2.16 antibody bound only to P cells of CBA ($I\text{-}A^k$) origin but not to P cells of BALB/c ($I\text{-}A^d$) or hybrid BDF_1 ($I\text{-}A^b$ and $I\text{-}A^d$) origin. Similarly, the 14.4-4S antibody bound to P cells of CBA ($I\text{-}E^k$) or BALB/c ($I\text{-}E^d$) origin but not to P cells of C57BL/6 origin.

Similar results were obtained whether indirect immunofluorescence was monitored by using fluorescence microscopy or the FACS, as shown in Fig. 1 along with the background fluorescence profile obtained with fluorescent anti-mouse Ig alone. The background was unaffected by treatment with CAS medium, and similar profiles were obtained with untreated cells, indicating that the background was due to low levels of endogenous P-cell fluorescence. These results suggested that CAS medium contained an Ia-inducing activity that was not present in W3-C medium.

Evidence that CAS Medium Induces the Synthesis of Ia Antigens. Several lines of evidence indicated that the Ia antigens appearing in the presence of CAS medium were directly synthesized by the P cells. One set of experiments addressed the possibility that the Ia antigens had been passively acquired from the spleen-conditioned medium. Using the monoclonal antibody 10-2.16, which binds to I-A^k antigens but not to I-A^b or I-A^d antigens, we found that CAS prepared from hybrid BDF_1 mice (I-A^b and I-A^d antigens) or BALB/c mice (I-A^d antigen) induced I-A^k antigens on CBA P cells (Fig. 2). Thus, the I-A^k molecules had been directly synthesized by the CBA P cells, as in these instances I-A^k antigens would not have been present in the CAS medium. In other experiments, we observed that an inhibitor of protein synthesis, cycloheximide, at a concentration of 5 μM, which did not affect the viability of P cells, blocked the appearance of Ia antigens in the presence of CAS medium, suggesting that the medium induced the synthesis of new Ia antigens on P cells.

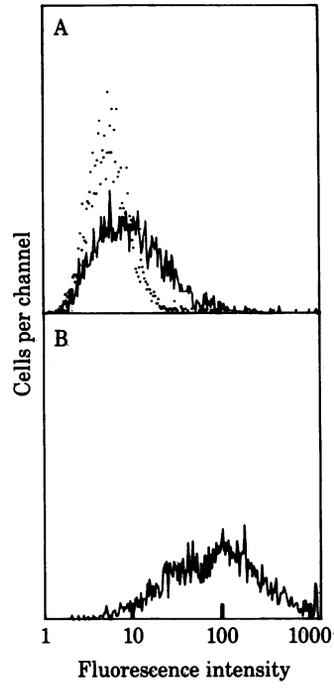

FIG. 1. Induction of Ia antigen expression by CAS medium. Ia-negative P cells were cultured without (*A*) or with (*B*) 3% CAS medium. After 48 hr, these cells were stained with monoclonal anti-I-A^k antibody 10-2.16 and fluorescein-conjugated sheep anti-mouse Ig (——) or with fluorescein-conjugated sheep anti-mouse Ig alone (·····) and analyzed on a FACS II. The intensity of fluorescence on a logarithmic scale is shown in arbitrary units.

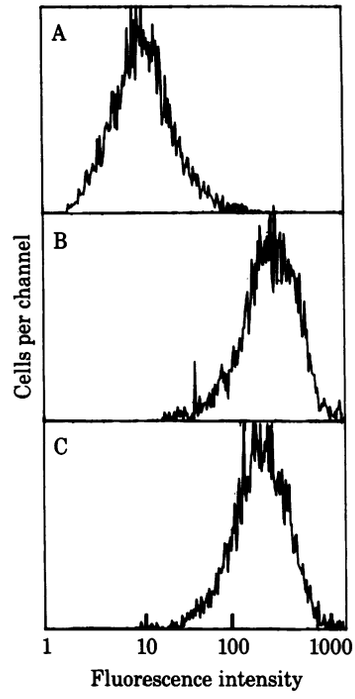

FIG. 2. The induction by CAS medium of Ia^k determinants on CBA P cells from BALB/c mice or hybrid BDF_1 mice. Ia-negative P cells were cultured in medium alone (*A*), or medium supplemented with BALB/c CAS medium (3%) (*B*), or BDF_1 CAS medium (3%) (*C*). Ia antigens were assayed at 48 hr as in Fig. 1. The titers of interferon in both BDF_1 and BALB/c CAS medium were 50 PRD_{50} per ml.

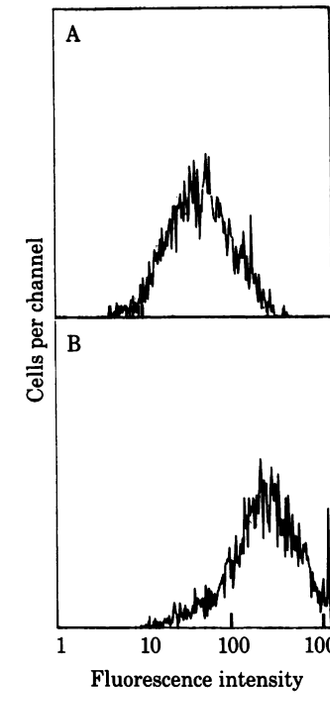

FIG. 3. Effect of CAS medium on the expression of H-$2K^k$ antigens. P cells were cultured without (*A*) or with (*B*) CAS medium (3%). After 48 hr, P cells were stained with monoclonal anti-H-$2K^k$ (11.4.1) and fluorescein-conjugated sheep anti-mouse Ig and analyzed with the FACS.

The phenomenon involved the induction of Ia expression on P cells, rather than the selective growth of low numbers of Ia-positive cells. A large percentage of the cells (50–70%) were Ia positive at 24 hr, and Ia antigens were inducible in the presence of mitomycin C (40 μg/ml) and hydroxyurea (4 mM), although cellular uptake of [^{3}H]thymidine was inhibited by >90%. Furthermore, pretreatment of P cells with monoclonal anti-Ia antibodies and complement failed to affect the induction of Ia-positive cells.

Effects of CAS Medium on Other Cell Surface Markers. In addition to the induction of Ia antigens on P cells by CAS medium, quantitative increases in the expression of H-$2K^k$ antigens were also detected with the FACS. The levels of indirect immunofluorescent staining with the monoclonal antibody 11.4.1 specific for H-$2K^k$ antigen (10) increased after incubation of CBA P cells with CAS medium (Fig. 3). Levels of some other markers were not increased after the incubation. These included the antigen Ly 5 and Fc receptors assayed either in a rosette assay or by using the FACS and monoclonal antibody directed against the Fc receptor. Receptors for the lectin peanut agglutinin, assayed with fluorescein-conjugated peanut agglutinin, in fact decreased.

Production of Ia-Inducing Activity and IFN-γ by T-Cell Hybridomas, a T-Cell Tumor, and Cloned T-Cell Lines. As a step in the analysis of the nature of the Ia-inducing activity, supernatants from several T-cell hybridomas and the thymoma EL-4 were tested for the ability to induce Ia antigens on P cells. Media conditioned by two T-cell hybridomas, 123 and T6, after stimulation by Con A failed to induce Ia antigens on P cells (Table 1), although these supernatants contained at least three distinct lymphokines, PSF, TCGF, and GM-CSF (11, 16–19). Also lacking Ia-inducing activity (Table 1) were the supernatants (*i*) from a T-cell hybridoma, T19-1, obtained after stimulation with Con A and containing GM-CSF (17), and (*ii*) from lymphoma EL-4, obtained after stimulation with 12-*O*-tetradecanoylphorbol 13-acetate and containing high levels of TCGF.

Supernatants of these cells also were tested for the presence of IFN and, in contrast to the CAS medium, IFN activity was not detected (Table 1). Furthermore, we found that supernatants obtained by antigenic stimulation of antigen-specific T-cell clones induced high levels of Ia antigens on P cells and that these supernatants also contained IFN-γ (Table 2). Previous experiments showed that the IFN-γ was synthesized by the antigen-specific T cells (13). Control supernatants (from the same T-cell clones cocultured with antigen-free lymph node cells) contained neither IFN-γ nor the Ia-inducing activity (Table 2).

Table 1. Effect of various conditioned media or partially purified factors on the induction of Ia antigens on CBA P cells

Additions to Ia^- P cells*	% Ia-positive cells (microscopy)	Mean fluorescence† (FACS)	Interferon titer, PRD_{50}/ml
Medium alone	<1	137	<5
Medium conditioned by			
Spleen cells (CAS)	87	1,734	50
T-cell hybridomas			
123	<3	256	<5
T6	<1	329	<5
T19.1	<1	209	<5
T lymphoma EL-4	<5	224	<5
Tumor WEHI-3B (W3-C)	<1	125	<5
Partially purified $TCGF^k$	<1	238	<5
Partially purified PSF^k	<1	150	<5

* Ia-negative P cells were cultured in the presence of the supernatants (5%) together with 0.1% W3-C medium 10 times concentrated for 3 days and stained for indirect immunofluorescence with the monoclonal anti-I-A^k antibody 10-2.16.

† Mean fluorescence intensity is in arbitrary units (mean channel number) on a logarithmic scale.

Table 2. Production of Ia-inducing activity by cloned T-cell lines

Supernatants	% Ia$^+$ cells (microscopy)	Mean fluorescence (FACS)	Interferon titer, PRD_{50}/ml
Oxazalone-specific T-cell clone			
Antigen-stimulated	85	2,036	100–200
Unstimulated	<1	235	<5
ABA-specific T-cell clone			
Antigen-stimulated	92	2,154	200
Unstimulated	<1	154	<5

ABA, azobenzene arsonate. Ia-negative P cells were cultured in the presence of unconcentrated supernatants (5%) prepared as described above from antigen-specific T-cell clones and containing the indicated amounts of antiviral activity (PRD_{50}/ml). After 3 days, P cells were stained with anti-Ia antibody 10-2.16 and analyzed by fluorescence microscopy or the FACS.

Partial Characterization of Ia-Inducing Activity. Preliminary characterization of the Ia-inducing activity has shown it to have characteristics identical to those of IFN-γ. It was sensitive to trypsin, stable to 56°C for 30 min, and nondialyzable. Most importantly, both the Ia-inducing activity and the antiviral activity in the CAS medium and the supernatants of the antigen-stimulated T-cell clones were inactivated by exposure to pH 2 (Fig. 4). The expression of H-2K^k antigens on P cells was increased also by these supernatants, and this activity was also sensitive to pH 2. Because both of the other interferons (IFN-α and IFN-β) are more stable to pH 2 (20, 21), this sensitivity to pH 2 indicated that the antiviral activity that correlated with the Ia-inducing activity was IFN-γ. A further parallel between IFN-γ and the Ia-inducing activity lay in our observations that medium conditioned by Con A-stimulated rat spleen cells but not medium conditioned by phytohemagglutinin-stimulated human leukocytes induced Ia antigens on P cells, although both supported the growth of T cells. Species specificity is also a characteristic of the antiviral activity of interferons (21).

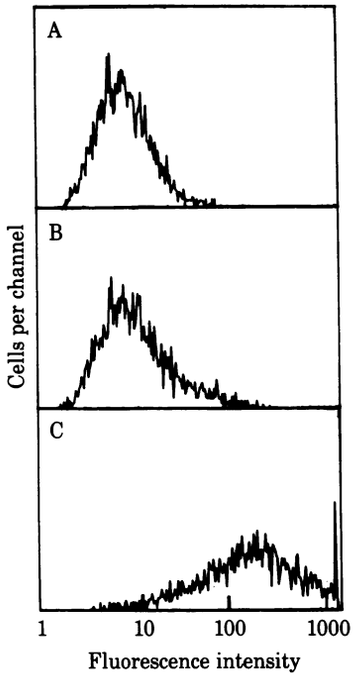

FIG. 4. Effect of treatment at pH 2 on the Ia antigen-inducing activity of supernatants of antigen-activated cloned T cells. Ia-negative P cells were cultured in medium alone (*A*) or medium supplemented with 3% supernatants treated at pH 2 (*B*) or pH 7 (*C*). The interferon titer of pH 2-treated supernatants was lower than 5 PRD_{50} per ml, whereas pH 7-treated supernatants contained 100–200 PRD_{50} per ml.

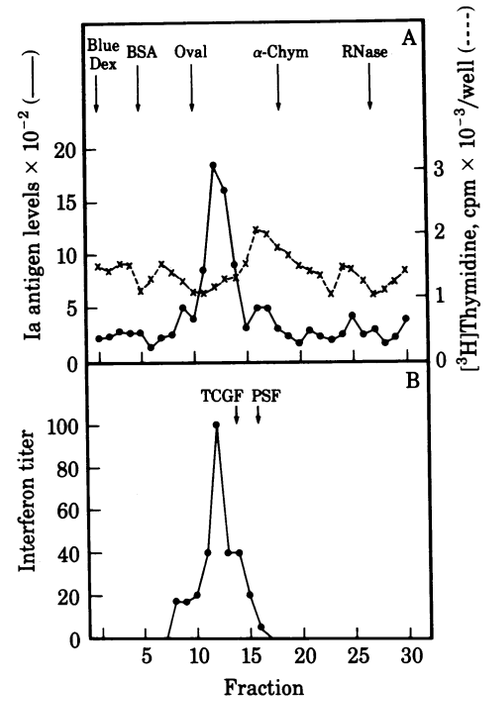

FIG. 5. Gel filtration of Ia-inducing activity. CAS medium 100 times concentrated (2 ml) was fractionated on a Sephadex G-75 superfine column as described (10). Calibration markers were: blue dextran (Blue Dex), M_r 2,000,000,000; bovine serum albumin (BSA), M_r 68,000; ovalbumin (Oval), M_r 43,000; α-chymotrypsin (α-CHYM), M_r 25,000; and ribonuclease (RNase), M_r 12,000. Ia-negative P cells were cultured for 48 hr in duplicate cultures containing the column fractions at 3%, supplemented with 0.1% W3-C medium 10 times concentrated to maintain viability. (*A*) Levels of Ia antigen expression were analyzed by FACS (——), and the growth of P cells was determined by the incorporation of [^{3}H]thymidine (---). (*B*) The titer of interferon in each fraction was assessed.

Fractionation of the Ia-inducing activity in CAS medium by Sephadex G-75 gel filtration showed that the Ia-inducing activity was eluted in a range (peak fractions 12–13) corresponding to an approximate M_r of 40,000–45,000 (Fig. 5*A*). The viral inhibitory activity due to IFN-γ was eluted in the same fractions (Fig. 5*B*). This is within the range of reported values of IFN-γ, which apparently can exist in more than one form with M_rs of 20,000–25,000 and 36,000–58,000 (21, 22). The peak of TCGF from the same column corresponded to a M_r of 30,000–35,000 and that of PSF, to a M_r of 30,000, in agreement with previous experiments (10, 18). The major peak of Ia-inducing activity thus coincided with IFN-γ, but not with TCGF or PSF. The activity increasing the expression of H-2K^k antigens on P cells also was found in the same fractions.

IFN-α/β, produced by the infection of mouse L cells with Newcastle disease virus (23), failed to induce Ia antigens on P cells. The maximal final concentration of IFN-α/β preparation used in these experiments had an antiviral activity of 40–80 PRD_{50} per ml. By contrast, the IFN-γ-containing supernatants of antigen-specific T-cell clones were active in inducing Ia antigens at concentrations corresponding to 2–4 PRD_{50} per ml.

DISCUSSION

We report here that Con A-stimulated spleen cells and cloned T-cell lines produce a molecule that increases the expression of class I (H-2K) and class II (Ia) major histocompatibility complex (MHC) antigens on P cells and that is identical in all respects tested with IFN-γ. Although the ultimate determination of the identity or otherwise of the Ia-inducing activity and IFN-

γ may await cloning of the relevant gene(s), the evidence for a close relationship is very strong. Class I MHC antigens occur on most cells, and the changes in levels of H-2K^k antigens seen in the present experiments were quantitative. The changes induced in levels of Ia antigens on P cells, on the other hand, were more remarkable because, in the absence of the Ia-inducing factor, both *I-A*- and *I-E*-encoded antigens were barely detectable on the majority of P cells. The finding that a T-cell-derived IFN-γ-like molecule has a pronounced effect on the expression of Ia antigens on these putative mast cell progenitors raises questions, both about the function of Ia antigens on cells not generally associated with antigen presentation and about the role of IFN-γ in regulating immunological and hemopoietic processes.

Ia antigens may act both by presenting antigens to T cells and by serving as targets for factors produced by helper and suppressor T cells. Either or both could be involved in the function of class II antigens on P cells and mast cells. A role in antigen presentation is suggested by the observations that (*i*) P cells are recognized both by allogeneic T cells and, in association with haptens, by syngeneic T cells (unpublished data) and (*ii*) at least *in vitro*, P cells can assist certain T-cell responses (7). On the other hand, the P cell, as a rapidly dividing cell with great capacity for self-renewal, probably corresponds to an immature stage of mast cell development and, in view of the reports of class II antigens on other hemopoietic progenitor cells (3–5), it could be speculated that class II antigens also serve some unknown function in hemopoiesis.

T-cell activation by Con A or antigens has been shown to be associated with the production of a soluble factor(s) that increases the level of Ia antigens on macrophages and macrophage-like tumor cell lines (24–26). Recent unpublished experiments with macrophage-like tumor cell lines suggest that this factor is IFN-γ. IFN-γ also may be instrumental in increasing the expression of Ia antigens on the epithelial cells in the skin and gut, both of which become Ia positive in the presence of T cells activated by allogeneic interactions (27, 28).

Other reports suggest that IFN-γ, in addition to its antiviral effects, has immunoregulatory roles. IFN-γ has been implicated in the induction of T-cell responses (29), the induction of macrophage killing (30), Fc receptor expression (31), and the induction of natural killer cell activity (32). IFN-γ has been reported to be more potent than IFN-α or IFN-β in suppressing antibody production (33) and in decreasing the growth of tumors in experimental animals (34). The IFN-γ used in those studies was not highly purified and, therefore, as with the present study, it is only possible to suggest an association of IFN-γ with the effects observed. In addition, crude (35) or partially purified (36) IFN-α has been shown to cause changes in the membrane proteins of cells, including the increased expression of HLA antigens and β-microglobulin; however, IFN-α/β, at least at the concentrations tested (40–80 PRD_{50}/ml) did not induce Ia antigens on P cells. Clearly IFN-γ-containing supernatants were much more potent in the present system.

The MHC antigens have an established role in defense against viral infection (1), and an increase in their levels by an IFN-γ-like molecule is a logical component of a coordinated pleiotropic antiviral response. However, because the release of IFN-γ is triggered by a multitude of nonviral antigenic stimuli (e.g., ref. 13), it is likely that the function of IFN-γ or a related product(s) has evolved to encompass other regulatory functions unrelated to antiviral responses. In view of the crucial role of products of the MHC in the initiation, expression, and regulation of immune responses, it is possible that regulation of products of the MHC may underlie many of the observed effects of IFN-γ on lymphoid and hemopoietic cells.

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