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Gamma Interferon Induction in Human Thymocytes Activated by Lectins and B Cell Lines

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Human thymocytes in culture synthesized small quantities of interferon (IFN) when stimulated by the lectins concanavalin A or phytohemagglutinin. IFN production by lectin-activated thymocytes was enhanced in the presence of live B lymphoblastoid cells, irradiated B lymphoblastoid cells, or the conditioned medium from B lymphoblastoid cell cultures. The IFN synthesized in mixed cultures had characteristics of IFN-γ, whereas the IFN synthesized by B lymphoblastoid cells alone could be identified as IFN-α on the basis of its neutralization with specific antisera and stability at pH 2. These findings indicate that human thymocytes in culture synthesize IFN-γ and that B lymphoblastoid cells and their products considerably stimulate IFN-γ synthesis by lectin-activated human thymocytes in culture. This stimulation was not diminished in the presence of antibodies to IFN-α, indicating that IFN-α production by B lymphoblastoid cells was not responsible for the stimulatory effect. Removal of adherent cells from thymocyte suspensions did not abrogate IFN-γ production.

Three major types of interferon (IFN), termed IFN-α (leukocyte), IFN-β (fibroblast), and IFN-γ (immune), can be distinguished on the basis of different antigenic, physicochemical, and biological properties (15). IFN-γ is typically produced by T cells after mitogenic stimulation with lectins or specific antigens (4, 5). In some instances, IFN-γ production by lectin-activated T cells is enhanced in the presence of autologous macrophages (6).

Very little is known about IFN synthesis by thymocytes, precursors of circulating T cells. In mice, Marcucci et al. (12) observed that steroid-resistant medullary thymocytes could be stimulated to produce IFN with phytohemagglutinin (PHA). In contrast, Stobo et al. (16) did not observe IFN production by murine steroid-resistant thymocytes in response to mitogens. There have been no reports of attempts to induce IFN production in human thymocytes.

The aim of this study was to determine whether human thymocytes synthesize IFN in vitro. We demonstrate that cultures of human thymocytes produce IFN-γ in response to concanavalin A or PHA. IFN-γ production by thymocytes could be enhanced by the addition of intact B lymphoblastoid cells, irradiated B lymphoblastoid cells, or the conditioned medium from B lymphoblastoid cell cultures. The fact that human thymocytes can be stimulated to produce IFN-γ shows that thymocytes can be induced to acquire one of the functions expressed by mature T cells. This implies either that the capacity to produce IFN is acquired at a relatively early stage of T cell ontogenesis or that IFN synthesis accompanies the induction of thymocyte differentiation in vitro.

MATERIALS AND METHODS

Preparation of cell suspensions. Thymocytes were isolated by teasing sections of human thymus obtained in the course of cardiac surgery, and the separated cells were suspended in RPMI 1640 medium. Two B lymphoblastoid cell lines maintained in permanent culture in RPMI 1640 medium supplemented with 10% donor calf serum, 2 mM glutamine, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 0.25 μg of amphotericin B per ml were used for these experiments. Line 1 was from a child deficient in purine nucleoside phosphorylase; line 2 was from a normal donor. The conditioned media were filtered through a 0.20-μm Nalgene filter and stored at −70°C. No mycoplasmas could be isolated from suspension cultures of lines 1 and 2 on standard mycoplasma agar.

Experimental conditions. Thymocytes and B lymphoblastoid cells were cultured in 96-well flat-bottomed plates containing RPMI 1640 medium supplemented with 5% heat-inactivated fetal calf serum (FCS), 2 mM glutamine, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 0.25 μg of amphotericin B per ml (200 μl per well) in a humidified atmosphere with 5% CO_2 at 37°C. Unless stated otherwise, the conditioned medium of each well was harvested after a 48-h incubation period and stored at −70°C until assayed for IFN. The titers measured reflect cumulative IFN secretion during the time of incubation.

Thymocyte depletion of adherent cells. For use in other experiments, thymocytes were depleted of ad-

TABLE 1. IFN synthesis by human thymocytes[a]

Expt.	No. of thymocytes per ml	B cell line (no. of cells per ml)	Lectin added	IFN titer	IFN type
A	6×10^6	None	None	4	
			Concanavalin A	128	γ
			PHA	64	
B	6×10^6	Line 1 (3×10^5)	None	8	
			Concanavalin A	2,560	γ
			PHA	3,072	γ
C	3×10^5	Line 1 (10^6)	None	64	
			Concanavalin A	128	
			PHA	320	
D	None	Line 1 (10^6)	None	32	α
			Concanavalin A	48	
			PHA	48	
E	None	Line 1 (3×10^5)	None	<4	
			Concanavalin A	32	
			PHA	8	
F	6×10^6	None	Conditioned medium	64	
			Conditioned medium + concanavalin A	512	γ
			Conditioned medium + PHA	192	γ
G	6×10^6	Line 2 (3×10^5)	None	4	
			Concanavalin A	320	γ

[a] Thymocytes were isolated and suspended in RPMI 1640 medium. Experimental conditions were as follows. Thymocytes and B lymphoblastoid cells, at the densities indicated (cells per milliliter) were cultured in RPMI 1640 medium supplemented with 5% heat-inactivated FCS, 2 mM glutamine, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 0.25 μg of amphotericin B per ml. Concanavalin A (10 μg/ml), PHA (5 μg/ml), or 100 μl of line 1 conditioned medium was added as indicated. Cells were cultured as described in the text and harvested after 48 h. The data shown were obtained from an experiment carried out with cultures prepared from one thymus. The type of IFN produced was determined by neutralization with antisera to IFN (see also Tables 3 and 5).

herent cells either by incubating them on a plastic surface for 3 h at 37°C or by passing them through a nylon wool column. The nonadherent thymocyte populations collected by either method were cultured in 2 ml of RPMI 1640 medium supplemented with 5% FCS and antibiotics in plastic Falcon flasks (25 cm^2) for various periods of time. Portions (200 μl) were removed for IFN assays, and fresh medium (200 μl) was added to keep the volume of culture fluid constant.

IFN assay. IFN titers were determined by microplate assay for antiviral activity with encephalomyocarditis virus as the challenging virus in GM-258 cells with trisomy 21, which are highly sensitive to IFN action. A laboratory standard of human IFN-γ was included with each assay (9). All titers were expressed in actual laboratory units without correction. The type of IFN produced was determined by neutralization with anitsera to IFN-α and IFN-β (8). Stability at pH 2 was tested by dialysis against HCl-glycine buffer (pH 2.0) for 18 h at 4°C. Before IFN assays, the pH of the sample was brought to 7.4 by further dialysis against minimal essential medium.

Antisera to IFN-α and IFN-β. Rabbits were immunized with human IFN-α (specific activity, 10^7 IU/mg of protein). The resulting antiserum neutralized homologous IFN-α and, to a lesser extent, human IFN-β. This antiserum was designated anti-IFN-α/β. Antiserum collected during the early stages of immunization neutralized IFN-α, but not IFN-β; this antiserum was designated anti-IFN-α. Antiserum specific for human IFN-β (anti-IFN-β) was prepared as described previously (10). Standard preparations of IFN-α, IFN-β, and IFN-γ used in the neutralization assay were prepared as described previously (10, 13).

RESULTS

IFN induction in thymocyte cultures and its stimulation by lectins and B cell lines. Unstimulated human thymocytes maintained in short-term culture produced at best very low levels of IFN (Table 1, experiment A). Exposure to the mitogen concanavalin A resulted in a 32-fold increase in IFN synthesis within 48 h, and exposure to PHA resulted in a 16-fold increase over the basal level. These results show that lectin activation does lead to IFN induction in

TABLE 2. Stimulation by live or irradiated B lymphoblastoid cells of IFN synthesis by lectin-activated human thymocytes[a]

Cultured cells	Lectin added	IFN titer after incubation for:			
		24 h	48 h	72 h	96 h
Thymocytes alone	None	<4	<4	<4	<4
Thymocytes alone	Concanavalin A	96	128	128	128
Thymocytes + B lymphoblastoid cells	None	4	24	48	64
Thymocytes + B lymphoblastoid cells	Concanavalin A	256	1,024	1,536	2,048
Thymocytes + irradiated B lymphoblastoid cells	None	6	32	48	24
Thymocytes + irradiated B lymphoblastoid cells	Concanavalin A	384	768	768	1,024
B lymphoblastoid cells alone	None	<4	<4	<4	<4
B lymphoblastoid cells alone	Concanavalin A	<4	<4	<4	<4
Irradiated B lymphoblastoid cells alone	None	<4	<4	<4	<4
Irradiated B lymphoblastoid cells alone	Concanavalin A	<4	<4	<4	<4

[a] Thymocytes at a density of 6×10^6 cells per ml were grown in short-term culture for up to 96 h (for conditions, see text). Where indicated, thymocytes were cocultured with 3×10^5 live B lymphoblastoid cells per ml (line 1) or with the same number of irradiated (4,800 rads) B lymphoblastoid cells (line 1). Concanavalin A (10 μg/ml) was added as indicated. The data shown were obtained from an experiment carried out on a specimen from one thymus. IFN titers represent cumulative IFN synthesis over the time indicated.

human thymocyte cultures. Next, we investigated the effect of B cell lines on IFN production. Coculture of thymocytes with either one of two human B cell lines enhanced IFN synthesis marginally or not at all (Table 1, experiments B and G). However, when thymocytes were exposed to two stimuli, activation with the lectin concanavalin A or PHA and coculture with B lymphoblastoid cells, the effect was synergistic, and IFN synthesis increased several hundredfold above that of unstimulated thymocytes (Table 1, experiment B). Some stimulation of IFN production was also obtained with a different B lymphoblastoid cell line, derived from another donor (Table 1, experiment G). The amount of IFN secreted into the culture medium in this experimental system was proportional to the number of thymocytes in culture and was dependent upon the ratio of thymocytes to lymphocytes (Table 1, experiments B and C). Maximum stimulation of IFN synthesis was observed when the ratio of thymocytes to B lymphocytes was 20 to 1 (Table 1, experiment B). On the other hand, if the ratio of thymocytes to lymphocytes was reversed so that the number of lymphocytes exceeded the number of thymocytes by approximately sevenfold, IFN synthesis was lower (Table 1, experiment C). If thymocytes were omitted from the cultures, small amounts of IFN were synthesized by B cells both in the absence and in the presence of concanavalin A or PHA (Table 1, experiments D and E). Conditioned medium from B lymphocyte cell lines could replace intact B cells in stimulating IFN production by thymocytes (Table 1, experiment F).

In a separate experiment we investigated whether the addition of X-ray-irradiated B lymphoblastoid cells also caused stimulation of IFN production (Table 2). Incubation of irradiated B lymphoblastoid cells with concanavalin A-activated thymocytes stimulated IFN production almost as effectively as did the addition of nonirradiated B lymphoblastoid cells. Irradiated B lymphoblastoid cells alone did not produce IFN.

Characterization of IFN produced by thymocytes and B cell lines. The IFN synthesized by lectin-activated thymocytes cocultured with B lymphoblastoid cells at a ratio of 20 to 1 was not neutralized by anti-IFN-α/β (Table 3). In addition, thymocyte IFN was destroyed at pH 2.0 (Table 4). It is therefore by definition IFN-γ. In contrast, IFN produced by the B lymphocyte line is IFN-α since it was neutralized by anti-IFN-α but not by anti-IFN-β and was stable at pH 2.0 (Table 5).

Enhancement of IFN-γ production in thymocytes as a result of the addition of B lymphoblastoid cells: mediation by IFN-α. It is well known that incubation of cells with IFN can result in the priming of subsequent IFN production (15).

TABLE 3. Effect of anti-IFN-α/β on IFN produced in thymocyte cultures[a]

IFN prepn	IFN titer after incubation with:	
	Control serum	Anti-IFN-α/β
Thymocyte IFN	128	128
Standard IFN-α	256	<4
Standard IFN-β	256	<4
Standard IFN-γ	512	512

[a] Conditioned medium from thymocytes (6×10^6 cells per ml) cultured with B lymphoblastoid cells (line 1, 3×10^5 cells per ml) and concanavalin A (10 μg/ml) was incubated with 1% FCS (control serum) or with 1% anti-IFN-α/β for 2 h at 37°C. IFN standards were incubated under the same conditions as the sample to be tested. Residual IFN activity was then assayed as described in the text.

TABLE 4. Inactivation of thymocyte-derived IFN at pH 2[a]

IFN prepn	IFN titer after dialysis at:	
	pH 7.4	pH 2.0
Thymocyte IFN	512	16
Standard IFN-α	4,096	4,096
Standard IFN-β	512	512
Standard IFN-γ	2,048	32

[a] Thymocytes were induced to synthesize IFN by incubating 6×10^6 cells per ml with B lymphoblastoid cells (line 1, 3×10^5 cells per ml) and concanavalin A (10 μg/ml). Portions of thymocyte IFN and of standard IFN preparations were dialyzed against either minimal essential medium at pH 7.4 or HCl-glycine buffer at pH 2.0 for 18 h at 4°C. All samples were assayed after further dialysis against minimum essential medium at pH 7.4 for 18 h at 4°C. For details, see the text.

Recently it was demonstrated that incubation of peripheral human lymphocytes with IFN-α or IFN-β enhanced the production of IFN-γ elicited by the addition of PHA (13). Since we found that the B cell line used produced small quantities of IFN-α, it is conceivable that the stimulatory effect of B lymphoblastoid cells is the result of a priming action mediated by IFN-α produced during coculture. To examine this possibility, cultures of thymocytes and B cells stimulated with concanavalin A were incubated in the presence or absence of anti-IFN-α/β. Any IFN-α or IFN-β produced in the cultures would be rapidly neutralized by the antiserum. As shown in Table 6, the stimulating effect of B lymphoblastoid cells on IFN-γ production was not abolished in the presence of anti-IFN-α/β. Therefore, this effect is not likely to be mediated by IFN produced by the B cell line.

Effect of adherent cells on IFN production by thymocytes. In view of the reported dependence of T lymphocytes upon adherent cells (macrophages) for the synthesis of IFN (5, 6), we examined whether human thymocytes depleted of adherent cells could still synthesize IFN when challenged by concanavalin A and lymphoblastoid cells (Table 7). Adherent cells were removed either by the incubation of thymocytes on plastic surfaces for 3 h at 37°C or by the passage of thymocytes through a nylon wool column. We found that mitogen-activated thymocytes depleted of adherent cells by incubation on a plastic surface and cocultured with B lymphoblastoid cells synthesized IFN as well as untreated thymocytes under the same experimental conditions. The passage of thymocytes through nylon wool columns, which results in the removal of an as yet unidentified population of adherent cells, also did not abrogate the capacity of activated thymocytes to synthesize IFN-γ under our experimental conditions.

DISCUSSION

Our findings indicate that human thymocytes produce IFN-γ when activated by concanavalin A or PHA and that this synthesis is enhanced by coculture with live or irradiated cells of a permanent B cell line or by the addition of conditioned medium from this B cell line. This is the first demonstration of IFN production by human thymocytes in culture. We have provided evidence that it is indeed the thymocytes and not the B lymphoblasts which synthesize IFN-γ in the mixed cultures, since stimulation with a lectin induced IFN-γ synthesis in thymocytes but not in B lymphoblasts. In addition, the cell-free conditioned medium of B lymphoblastoid cells also resulted in a stimulatory effect on the induction of IFN-γ synthesis by thymocytes. Earlier studies showed that IFN produced spontaneously by B lymphoblastoid cell lines has characteristics of IFN-α (9, 11). Our data confirm these findings.

Earlier studies showed that many transformed cell lines have the capacity to induce IFN production in cultures of peripheral lymphocytes (18). NK cells appear to be highly active in producing IFN in response to allogeneic and syngeneic tumor cells (17). Others have concluded that IFN production in response to tumor cells is the function of B cells (19). IFN produced in response to tranformed cells was found to be IFN-α (17). More recently, Beck et al. (1) and Birke et al. (2) showed that IFN-α induction by cell lines is often due to the presence of mycoplasma contamination rather than to the transformed character of the cells. This finding

TABLE 5. Antigenic properties of IFN produced by B lymphoblastoid cells[a]

Sample	IFN titer after incubation with:		
	Control serum	Anti-IFN-α	Anti-IFN-β
B lymphoblastoid cell medium	16	<4	16
Standard IFN-α	512	<4	512
Standard IFN-β	512	512	<4

[a] The conditioned medium from B lymphoblastoid cells (line 1) and the standard IFN preparations were incubated with 1% FCS (control serum) or with an excess of anti-IFN-α or anti-IFN-β for 2 h at 37°C. Residual IFN activity was assayed as described in the text.

TABLE 6. Effect of the addition of anti-IFN-α/β during coculture of thymocytes and B lymphoblastoid cells[a]

Expt. conditions	IFN titer at 48 h in the presence of:	
	Control serum	Anti-IFN-α/β
Thymocytes alone	<4	<4
Thymocytes + concanavalin A	128	256
Thymocytes + B lymphoblastoid cells	<4	<4
Thymocytes + B lymphoblastoid cells + concanavalin A	512	1,024
B lymphoblastoid cells alone	<4	<4
B lymphoblastoid cells + concanavalin A	<4	<4
Standard IFN-α[b]	256	<4
Standard IFN-β[b]	512	<4
Standard IFN-γ[b]	1,024	512

[a] Thymocytes (6×10^6 cells per ml) were cultured in complete RPMI 1640 medium. Concanavalin A (10 μg/ml) or lymphoblastoid cells (line 1, 3×10^5 cells per ml) were added where indicated. Normal rabbit serum (control serum, diluted 1:200) or rabbit anti-IFN-α/β (diluted 1:200) was added at the time cultures were initiated. Culture fluids collected at 48 h were assayed for IFN activity.

[b] To check the potency and specificity of the IFN antisera, standard IFN preparations were incubated for 1 h at 37°C with the same control serum diluted 1:200 and IFN antisera diluted 1:200. Residual IFN activity was then determined.

raises the question of whether, in our experiment, stimulation of IFN production in thymocytes by B lymphoblastoid cells could also be due to contamination with mycoplasmas or, possibly, to some viral contamination. However, this possibility seems most unlikely. First of all, IFN produced in response to mycoplasmas or viruses would likely be IFN-α (or, perhaps, IFN-β) but not IFN-γ. Furthermore, our cell lines were free of mycoplasmas, and the conditioned medium used in our experiment was filtered through a 0.20-μm Nalgene filter. Beck et al. (1) reported that the IFN-inducing agents of murine cell lines were mycoplasmas which did not pass through a 0.22-μm filter.

TABLE 7. IFN synthesis by human thymocytes depleted of adherent cells[a]

Cells	IFN titer after incubation for:			IFN type[b]
	24 h	48 h	120 h	
Untreated thymocytes	32	256	1,024	γ
Thymocytes not adherent to plastic surfaces	64	256	512	
Thymocytes not adherent to nylon wool	32	32	512	

[a] For experimental details, see the text.

[b] See Table 1, footnote *a*.

The nature of the stimulus responsible for the IFN-γ-enhancing activity associated with B lymphoblasts and their products requires further characterization. One possibility is that the stimulation of IFN-γ production by B lymphoblastoid cells may be analogous to the enhancement of interleukin 2 production by human peripheral T lymphocytes during coculture with a B lymphocyte cell line (3, 7). It was suggested that the enhanced production of interleukin 2 in the presence of B cells may be due to a mixed lymphocyte reaction.

The majority of thymocytes are not immunocompetent and are, therefore, less likely to respond to stimulation with lectins or allogeneic B lymphocytes than mature T lymphocytes (14). Thymocytes apparently require two signals for maximum IFN synthesis: a lectin and the product(s) of a B cell line. The response to both signals exceeds the sum of the responses to either signal alone. Our observations clearly indicate that, despite their apparent lower responsiveness, human thymocytes can be induced to acquire an immunoregulatory function usually associated with mature T lymphocytes, namely the production of IFN-γ.

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