

Effect of cytokines on HLA-DR and IL-1 production by a monocytic tumour, THP-1

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SUMMARY

The monocytic tumour, THP-1, expresses many of the properties of monocytes, both by cell surface staining and its capacity to produce monokines. It was used as a source of homogenous monocytic cells as a model to determine whether a variety of highly purified or recombinant cytokines could induce HLA-DR expression and the production of interleukin-1 (IL-1). Interferon-gamma (IFN- γ) alone induced HLA-DR. Tumour necrosis factor (TNF), lymphotoxin (LT) and granulocyte-macrophage colony-stimulating factor (GM-CSF) alone were able to induce IL-1 but not HLA-DR. When IFN- γ was combined with TNF, induction of HLA-DR and IL-1 was enhanced in a synergistic manner. These effects were detectable at a pretranslational level as synergistic effects were observed on DR α mRNA and IL-1 β mRNA levels. The results demonstrate the specificity of IFN- γ as the inductive stimulus for HLA-DR expression by THP-1 cells. As IFN- γ and TNF are products of activated T cells, the synergistic role for these molecules in macrophage activation is discussed.

INTRODUCTION

The induction of immune responses depends on the recognition by T cells of antigenic determinants in association with MHC class II molecules (termed HLA-DR, DP and DQ in man; reviewed by Schwartz, 1985). The expression of class II genes is limited primarily to cells of the immune system: macrophages, B lymphocytes, dendritic cells and activated T lymphocytes. Aberrant expression of HLA class II has been noted in human autoimmune diseases (Bottazzo *et al.*, 1983) and it has been demonstrated that this may lead to the chronic stimulation of autoreactive T cells (Londei, Bottazzo & Feldmann, 1985).

The efficiency of antigen presentation depends partly on the density of class II molecules on the cell surface. The capacity of IFN- γ to induce MHC class II on a variety of cell types has been well documented (Collins *et al.*, 1984; Wong *et al.*, 1984; Todd *et al.*, 1985). In a number of studies, compounds other than IFN- γ have been shown to induce MHC antigens. TNF has been

reported to induce Ia in a murine macrophage tumour line (Chang & Lee, 1986) and is an effective signal in combination with IFN- γ to induce HLA-DR on human pancreatic endocrine cells, which are unresponsive to IFN- γ alone (Pujol Borrell *et al.*, 1986, 1987). E⁻ cells displayed elevated class II expression after treatment with EGF and PDGF (Acres, Lamb & Feldmann, 1985), and thyroid follicular cell class II is augmented (perhaps induced) by thyroid stimulatory hormone (Todd *et al.*, 1987).

Soluble factors produced by antigen-presenting cells are also of importance in stimulating T-cell activation. The best characterized of these is IL-1. However, a role for accessory cell-derived TNF and IL-6 is also envisaged based on recent reports that these cytokines can also act as co-factors in T-cell proliferation assays (Yokota, Geppert & Lipsky, 1988; Lotz *et al.*, 1988). Induction of IL-1 is regulated by multiple signals, such as bacterial endotoxins or phagocytic stimuli (Dinarello, 1984), other cytokines such as TNF (Dinarello *et al.*, 1986) and by IL-1 itself (Philip & Epstein, 1986).

The objective of this study was to test a wide variety of cytokines for their ability to induce HLA-DR and IL-1 in a homogenous population of cells and hence gain insights into the signals required to generate effective antigen-presenting function. Our data suggest a dissociation of the signals required for antigen presentation and those required for amplification of the immune response.

MATERIALS AND METHODS

Cytokines: sources and specific activities

Highly purified murine EGF was purchased from Gibco BRL

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Abbreviations: E⁻, erythrocyte rosette-depleted mononuclear cells; EGF, epidermal growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN- γ , interferon-gamma; IL-1, interleukin; LT, lymphotoxin; M-CSF, macrophage colony-stimulating factor; PBMC, peripheral blood mononuclear cells; PDGF, platelet-derived growth factor; TGF α , transforming growth factor α ; TGF β , transforming growth factor β ; TNF, tumour necrosis factor.

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(Paisley, Renfrewshire); 5 ng/ml EGF yielded half maximal activity in a normal rat kidney cell line proliferation assay. PDGF from porcine platelets (specific activity 30,000 U/mg) was purchased from BioProcessing Ltd, Consett, Durham. *E. coli*-derived TGF α , and TGF β were supplied by Genentech Inc. (San Francisco, CA); 1 mg TGF α had an activity of 0.55 mg EGF equivalents. Both EGF and TGF α were active in a human thymocyte cell growth assay. TGF β was assayed by its ability to inhibit IL-2-driven T-cell mitogenesis and was active at picomolar concentrations. *E. coli*-derived TNF, LT and IFN- γ were obtained from Genentech and Dr G. Adolf Boehringer, Ingelheim, FRG, IFN- γ was also purchased from Amersham International (Amersham, Bucks). The specific activity of IFN- γ was $\sim 2 \times 10^7$ U/mg as assayed by viral inhibition in A549 cells. TNF had a specific activity of 5×10^7 U/mg, and LT a specific activity of 1.2×10^8 U/mg, as assayed on L929 cells. *E. coli*-derived GM-CSF and IL-3, in the form of a COS cell supernatant, were supplied by Dr Gordon Wong (Genetics Institute, Cambridge, MA). GM-CSF activity was 4.7×10^6 colony-forming units (CFU)/mg. IL-3 displayed half maximal bioactivity at a 1/21,900 dilution and was saturating at a 1/2500 dilution. M-CSF (CSF-1) was supplied by Dr P. Ralph (Cetus, Emeryville, CA) and had a specific activity of 10^6 U/ml. IL-1 α was supplied by Dr P. Lomedico (Hoffman La-Roche, Nutley, NJ). It had an activity of 10^9 U/mg in the D10 T-cell proliferation assay and was routinely assayed in our laboratory. IL-2 was provided by Dr J. Hamuro, Ajinomoto Inc. (Kawasaki, Japan) and had a specific activity of 1.2×10^7 U/mg. IL-4 was supplied by Immunex Corporation (Seattle, WA). It had 10^8 U/mg in the anti- μ comitogenesis assay on B cells. Both IL-2 and IL-4 were active in T-cell proliferation assays in our laboratory. IL-6 was provided by Dr T. Kishimoto (Osaka University, Japan). It had an activity of 5×10^6 U/mg, as assayed by hybridoma growth. Where appropriate endotoxin levels were determined by limulus amoebocyte gelation (Sigma, Poole, Dorset) and found to be less than 30 pg/ml.

Rabbit antisera against human TNF and LT were the gift of Dr G. Adolf (Boehringer Institute, Vienna).

Cells

Mycoplasma-free THP-1 cells were generously provided by Dr K. Matsushima (NIH, Fredrick, MD). These cells are derived from a 1-year-old individual with acute monocytic leukaemia (Tsuchiyama *et al.*, 1980). THP-1 expresses Fc receptors, C3 receptors and can be induced to produce IL-1, TNF and PDGF. With phorbol esters it adheres to plastic and acquires a macrophage morphology.

THP-1 cells were cultured in complete RPMI-1640 containing 10% FCS. To study the effect of recombinant mediators, 24-well tissue culture plates were set up containing 1 ml of cells plus 10 μ l of test factor at varying dilutions, to provide the final concentration desired.

¹²⁵I labelling

Sheep IgG anti-mouse IgG, which had been adsorbed on a human IgG affinity column and affinity purified on a mouse IgG-Sepharose 4B column, was labelled by the Iodogen method (Pierce Chemical Co., Rockford, IL) as described by the manufacturer.

Radioimmunoassay for HLA-DR

After 2–6 days in culture, cells were transferred to 12 \times 75 mm tubes and washed with 1% bovine serum albumin (BSA)-RPMI containing 0.1% sodium azide and resuspended in RPMI-1640 containing 20% horse serum (to block Fc receptors) and 0.1% azide, to a final concentration of 5×10^5 cells/ml; 0.1 ml of cells were added to each well of a V-bottom 96-well microtitre plate. Monoclonal antibody to HLA-DR L243, obtained from the ATCC (Bethesda, MD; HB55), which had been purified on protein A-Sepharose, was added to test wells at a final concentration of 5 μ g/ml. A mouse γ 2a monoclonal antibody to rat astrocyte surface glycoprotein which does not cross-react with human cell surface antigens was used as a control. The plates were incubated overnight at 4° and washed twice with wash medium and resuspended in assay medium. ¹²⁵I-labelled sheep anti-mouse IgG was added at a final concentration of 500 ng/ml (the optimal concentration) and cells were incubated for 4 hr at room temperature. The cells were washed three times, resuspended to 100 μ l, transferred to tubes and counted in an LKB gamma-counter. Samples were set up in triplicate.

Filter hybridization

Slot blots of cytoplasmic RNA were made using the method described elsewhere (Turner, Londei & Feldmann, 1987) and filters were prehybridized, probed and washed to high stringency as described previously (Turner *et al.*, 1987). The IL-1 β probe (Auron *et al.*, 1984) was provided by Dr P. Lomedico (Hoffman La Roche). The DR α cDNA was a 500 bp insert from pDRH-2 (Lee, Trowsdale & Bodmer, 1982). The cDNA probe for 7B6 mRNA was a 708 bp Pst I-Dra I fragment containing the Pst I-Dra I region from pBR322; this was the gift of Professor U. Torelli (University of Modena, Italy) and detects a cell cycle-independent species of mRNA. Autoradiographs were scanned using a Joyce Loebel Chromoscan-3, and peak results were integrated; final values were normalized to 7B6.

Assay for IL-1

IL-1 levels were measured using the thymocyte co-stimulator assay (Paetkau *et al.*, 1976). An internal standard of recombinant human IL-1 α (Hoffman La Roche) was routinely used in this assay.

RESULTS

Fourteen highly purified or recombinant DNA-derived cytokines were tested singly over a large dose range for their ability to induce DR expression as assessed by specific RIA and IL-1 production, as assessed by thymocyte co-mitogenesis. The results of this study are summarized in Table 1.

IFN- γ was the only cytokine which by itself could induce significant HLA-DR expression. The response to IFN- γ was both dose (Fig. 1) and time dependent; HLA-DR was elevated by 24 hr, peaked at 48–72 hr and then waned (data not shown). IFN- γ did not induce IL-1 activity above background levels, even when tested at a concentration of 1000 U/ml (Fig. 2a).

TNF and LT have been reported to augment IA expression on murine WEHI-3B cells (Chang & Lee, 1986), but by themselves were without effect on THP-1 HLA-DR expression (Fig. 1). Both TNF and LT were able to induce IL-1 production (Fig. 2).

GM-CSF failed to stimulate HLA-DR but induced the

Table 1. IFN- γ is the dominant stimulus for HLA-DR but not IL-1 expression by THP-1 cells

Cytokine	Dose range tested	HLA-DR	IL-1
IFN- γ	0.1–1000 U/ml	+	–
EGF	1 pg–100 ng/ml	–	–
TGF α	100 pg–1 μ g/ml	–	–
GM-CSF	0.1–1000 U/ml	–	+
M-CSF	0.1–100 U/ml	–	–
TGF β	1–1000 pg/ml	–	–
PDGF	0.01–3 U/ml	–	–
IL-1	0.1–100 U/ml	–	NT
IL-2	0.1–100 U/ml	–	NT
IL-3	1:1,000,000–1:1000	–	–
IL-4	0.1–10000 U/ml	–	–
IL-6	0.01–100 U/ml	–	NT
TNF	0.1–1000 U/ml	–	+
LT	0.1–1000 U/ml	–	+

Summary of cytokine effects on HLA-DR and IL-1 production by THP-1 cells. Each cytokine was tested over a large dose range as indicated in the table. HLA-DR expression was assessed by radioimmunoassay and IL-1 production by thymocyte co-mitogenesis assay as described in the Materials and Methods.

+ indicates a positive effect; – indicates no measurable change was observed; NT indicates not tested.

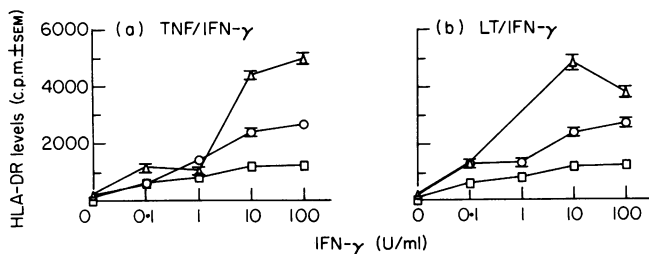


Figure 1. (a) Induction of cell surface HLA-DR on THP-1. THP-1 was incubated for 24 hr with various doses of IFN- γ by itself (squares) or with IFN- γ + TNF 0.1 U/ml (circles) or TNF 1 U/ml (triangles). HLA-DR levels were assessed by radioimmunoassay as described in the Materials and Methods. (b) THP-1 was incubated with various doses of IFN- γ alone (squares), IFN- γ with LT 0.1 U/ml (circles) or LT 1 U/ml (triangles) and HLA-DR levels determined by RIA.

production of IL-1 (Fig. 2). All other cytokines tested failed to induce HLA-DR (Table 1). IL-1 production was not induced by any of the other cytokines tested (Table 1); IL-1, IL-2 and IL-6 were not tested for their capacity to induce IL-1 because carry-over of these mediators stimulated thymocyte proliferation.

Studies of the induction of IL-1 in different laboratories has often given conflicting results due to contamination of media and other reagents with bacterial endotoxins. In order to exclude endotoxin effects we screened the materials used in this study for endotoxin using the limulus assay, and rejected reagents containing LPS detectable in that assay. As shown in

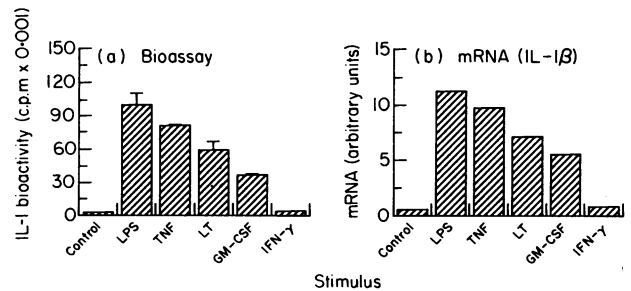


Figure 2. (a) THP-1 was cultured with medium alone, LPS 1 μ g/ml, TNF 2500 U/ml, LT 2500 U/ml, GMCSF 2500 U/ml, IFN- γ 1000 U/ml. THP-1-conditioned medium was collected after 24 hr. The effect of cytokines on IL-1 release was assessed by thymocyte comitogenesis. (b) Cytoplasmic mRNA was extracted and blotted onto nitrocellulose, IL-1 β mRNA levels were determined by scanning densitometry of autoradiograms and integral values normalized to the control probe 7B6.

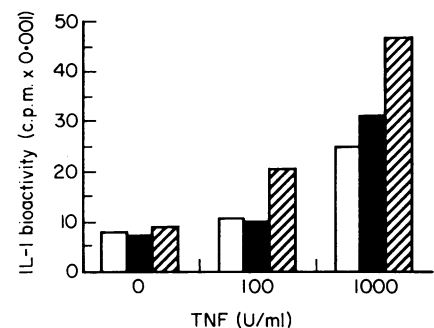


Figure 3. Enhancement of IL-1 production by the combination of TNF and IFN- γ . THP-1 was incubated for 24 hr with TNF alone (open bars) and either TNF + 100 U/ml (filled bars) or 1000 U/ml (hatched bars) IFN- γ . Culture supernatants were assayed for IL-1 bioactivity as described in the Materials and Methods.

Table 2, heat inactivation (65 $^{\circ}$, 25 min) was sufficient to abolish IL-1 induction by LT, TNF and GM-CSF but not LPS, and polymyxin B reduced IL-1 induction by LPS but not by TNF or LT and only slightly by GM-CSF. Finally, neutralizing polyclonal antisera against TNF and LT specifically inhibited IL-1 induction by the appropriate cytokine (Table 2).

We next studied the capacity of the combination of IFN- γ and TNF to induce HLA-DR and IL-1. Synergistic induction of HLA-DR was observed after 48 hr of culture when 0.1 or 1 U/ml TNF or LT was combined with IFN- γ (Fig. 1). This effect was apparent at a concentration of 0.1 U/ml IFN- γ and increased in a dose-dependent manner (Fig. 1). IL-1 activity was measured in THP-1-conditioned media and also found to be increased in a synergistic manner by the combination of TNF with IFN- γ treatment (Fig. 3).

Cytoplasmic RNA was extracted from cultures of THP-1 that had been treated with the cytokines used to induce IL-1 activity, and levels of IL-1 β RNA determined (Fig. 2b). A good agreement was found to exist between levels of IL-1 β mRNA and IL-1 bio-activity (Fig. 2a), suggesting a major mechanism of IL-1 induction by these cytokines is by increasing the steady-state levels of IL-1 mRNA. Although THP-1 produces IL-1 α mRNA, its levels are at least 100-fold less than IL-1 β , and IL-1 β comprises >90% IL-1 bio-activity secreted by THP-1 (Turner,

Table 2. Specificity of IL-1-inducing agents

Treatment	Inducing agent			
	TNF (1000 U/ml)	LT (1000 U/ml)	LPS (25 µg/ml)	GM-CSF (2500 U/ml)
None	48,320 ± 3967	35,936 ± 2902	52,306 ± 5003	23,428 ± 2819
Anti-TNF	14,306 ± 987 (<i>P</i> < 0.02)	33,583 ± 3055	50,982 ± 4387	ND
Anti-LT	49,960 ± 1023	10,091 ± 974	48,306 ± 3906	ND
Heat	12,081 ± 651	11,380 ± 1007	56,041 ± 6834	8405 ± 1629
Inactivation	(<i>P</i> < 0.02)	(<i>P</i> < 0.02)		(<i>P</i> < 0.05)
Polymyxin B	42,016 ± 5032	39,483 ± 5438	22,830 ± 980 (<i>P</i> < 0.05)	17,076 ± 780

To ensure that the induction of IL-1 was not due to the presence of endotoxin contamination in the cytokine preparations the effects of preincubation with the appropriate neutralizing antibody (where available), of heat inactivation, and treatment with polymyxin B, was determined. The effects of endotoxin can be inhibited by polymyxin B but not by the other treatments. For comparison the effects of these treatments on LPS-induced IL-1 are shown. Statistically significant differences between the treated and untreated groups were determined by Student's *t*-test. Proliferation due to PHA alone did not exceed 7506 ± 798 c.p.m.

Chantry & Feldmann, 1988). IFN- γ by itself consistently caused small but significant increases in IL-1 β mRNA, but did not induce IL-1 activity. When IFN- γ was added together with TNF, IL-1 β mRNA levels were increased and the effect was more than additive (Fig. 4a). DR α mRNA levels were increased by IFN- γ alone (Fig. 4b) and TNF, which had no effect by itself, enhanced DR α mRNA levels (Fig. 4b). These observations suggest that the mechanism of synergy between TNF and IFN- γ is partly mediated by pretranslational events.

DISCUSSION

Macrophage activation is a common feature at sites of immune/inflammatory responses. Activated macrophages express HLA class II (which is essential for antigen presentation) and elaborate a number of inflammatory mediators, including reactive oxygen metabolites and cytokines such as IL-1 and TNF. Over-expression of HLA class II is often associated with autoimmune diseases (Botazzo *et al.*, 1983), and the products of activated macrophages are readily detectable at autoimmune sites (Buchan *et al.*, 1988). Furthermore both IL-1 and TNF have been implicated in the tissue damage that occurs in autoimmune disease (Mandrup-Poulsen *et al.*, 1987).

The findings presented here suggest that the expression of HLA class II by macrophages can be dissociated from the production of the cytokine IL-1. No single mediator alone could induce HLA-DR and IL-1 simultaneously. Of the 14 mediators tested, IFN- γ alone was a dose- and time-dependent inducer of class II expression on THP-1 cells. IFN- γ is produced exclusively by cells of the lymphocytic lineage (Epstein, 1981), thus class II expression appears to be tightly regulated, its levels being dependent on a product of cells capable of recognizing and responding to antigen. Significantly, LT, TNF α and IFN- γ may be produced by the same T cell (Turner *et al.*, 1987). While TNF or LT were unable to induce HLA-DR *per se*, both could synergistically enhance the expression of HLA-DR in response to IFN- γ . The inability of TNF alone to induce HLA-DR is in contrast to other studies using tumour cell lines (Chang & Lee, 1986; Pfizenmaier *et al.*, 1987) but consistent with studies using non-transformed cell lines (Pujol-Borrel *et al.*, 1987; Lapierre, Fiers & Pober, 1988). We conclude that the products of activated T cells alone are capable of initiating and amplifying the expression of surface molecules and soluble mediators critical for antigen presentation.

It is of interest that different results were obtained with respect to macrophage activation leading to IL-1 production. In

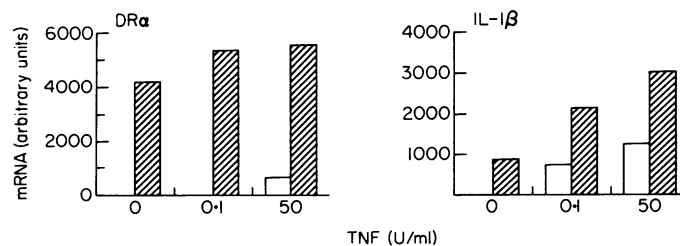


Figure 4. Effect of TNF and IFN- γ on DR α and IL-1 β mRNA. THP-1 was treated with different doses of TNF either in the presence (hatched bars) or absence (open bars) of 100 U/ml IFN- γ for 24 hr. RNA levels were determined by autoradiography followed by scanning densitometry, integral values were normalized to control probe 7B6. (a) Hybridization with DR α cDNA; (b) the blot was rehybridized with IL-1 β -specific cDNA.

the absence of endotoxin, IFN- γ does not induce IL-1 production but it is a potent enhancer of LPS-induced IL-1 production (Newton, 1985). We were careful to exclude the potential complications caused by endotoxins when studying IL-1 production. Media were tested using the limulus assay; inducers of IL-1 were shown to be neutralized by specific antisera or incubation at temperatures (65°, 25 min), which inactivated the cytokine but not LPS. Finally polymyxin B was shown to inhibit IL-1 induction by LPS, but not by the cytokines. Thus IFN- γ itself is incapable of triggering IL-1 production by THP-1 cells but synergises with TNF which alone is a potent IL-1 inducer. IFN- γ alone did cause small increases in the levels of IL-1 β -specific mRNA, consistent with the notion that the effect of IFN- γ may be to increase mRNA transcription or stability (Collart *et al.*, 1986). In our hands IFN- γ does not induce IL-1 production by freshly isolated monocytes but we have observed that this is highly dependent on the purity of the monocyte population as PBMC from the same donors can be induced by IFN- γ to express IL-1 mRNA and protein. These findings reflect the difficulty of obtaining pure populations of monocytes and may explain the discrepancy between the findings presented here and those of Acres *et al.* (1985) who showed that EGF could induce class II expression on E⁻ cells (a mixture of B cells and monocytes with a small number of residual T cells). Our findings and the work of others (Gerrard *et al.*, 1987) strongly support the view that macrophage activation as judged by the production of effector molecules such as IL-1 can take place independently of class II expression and hence the capacity for antigen presentation. This independence of gene expression may well reflect roles of IL-1 which are unrelated to antigen presentation. Such effects may include angiogenesis and fibroblast proliferation that contribute to wound healing or fever and cachexia in immunosuppressed cancer patients.

The ability of TNF and GM-CSF to induce IL-1 is noteworthy, since unlike IFN- γ and LT, which are exclusively products of activated lymphocytes, TNF and GM-CSF are produced by a variety of different cell types (Munker *et al.*, 1986; Koeffler *et al.*, 1987; Turner *et al.*, 1987; Hensel *et al.*, 1987) including the THP-1 cell line itself (M. Turner and D. Chantry, unpublished observations). This indicates the potential for paracrine activation of macrophage IL-1 production by such cells as endothelial cells and fibroblasts (Howells, Chantry & Feldmann, 1988). This phenomenon may contribute to the recruitment of macrophages into inflammatory tissues such as the rheumatoid synovium or the atherosclerotic plaque. Autocrine stimulation of IL-1 production may also be a feature of macrophage regulation, and could represent an amplification mechanism or a feedback loop leading to chronic stimulation.

In this study we have not addressed the question of inhibitors of class II expression; recent reports suggest type-1 interferons can antagonize IFN- γ -induced class II expression (Lapierre *et al.*, 1988) and that TGF β may inhibit class II expression (Czarniecki *et al.*, 1988). The ability of THP-1 to respond to multiple positive and negative signals suggests THP-1 cells may represent a useful model system to study the molecular basis of the differential regulation of HLA class II and the production of monokines such as IL-1 and TNF.

Note added in proof

Recent studies indicate GM-CSF may induce class II expression

on non-transformed human monocytes (D. Chantry, Brennan, M. Turner and M. Feldman, manuscript in preparation).

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