

CYTOKINES IN CHRONIC INFLAMMATORY ARTHRITIS

IV. Granulocyte/Macrophage Colony-stimulating Factor-mediated

Induction of Class II MHC Antigen on Human Monocytes:

A Possible Role in Rheumatoid Arthritis

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Type A synoviocytes and synovial tissue macrophages express high levels of class II MHC proteins (also known as Ia) on their surface in rheumatoid arthritis (RA)¹ (1). Although the mechanism of Ia induction is not known, IFN- γ has been proposed as the major factor responsible for this observation (2). However, we have recently demonstrated that the rheumatoid joint contains very low levels of IFN- γ that are unlikely to account for the high class II antigen expression (3, 4). In addition, an Ia-inducing factor was observed in rheumatoid joint samples that was not neutralized by antibody to IFN- γ (3).

These data suggest that a factor other than IFN- γ is responsible for the activated phenotype of type A synoviocytes and macrophages in RA synovium. In our studies on the cytokine profile in RA, we have recently demonstrated granulocyte/macrophage CSF (GM-CSF) in rheumatoid synovial effusions and in supernatants of cultured RA synovial tissue cells (5). GM-CSF induces surface Ia expression in murine macrophages (6-8), but similar activity has not been reported in humans. To examine the possibility that GM-CSF is responsible for the high level of surface HLA-DR on macrophage lineage cells in the RA synovium, we studied the effect of GM-CSF and a panel of other cytokines on human monocyte Ia expression. This cell type was selected because of its easy accessibility and the fact that tissue macrophages and type A synoviocytes are probably derived from bone marrow precursors of the monocyte lineage (9). Of the cytokines tested in these experiments, only GM-CSF and IFN- γ were potent Ia inducers. Although, the Ia-inducing factor produced by RA synovial tissue in vitro was not neutralized by anti-IFN- γ antibodies, it was blocked by specific antibody to GM-CSF. Because we have also recently measured significant levels of GM-CSF in rheumatoid joint samples (5), it is likely that this cytokine is an important Ia-inducing factor in the rheumatoid synovium.

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¹ *Abbreviations used in this paper:* GM-CSF, granulocyte/macrophage CSF; MFC, mean fluorescence channel; RA, rheumatoid arthritis.

Materials and Methods

Cell Preparation. Blood was drawn from healthy volunteers into heparinized syringes and diluted 1:1 with PBS. Mononuclear cells were separated by centrifugation on a discontinuous Ficoll-Hypaque density gradient at 300 *g* for 40 min, washed, and resuspended in RPMI 1640 with 10% heat-inactivated FCS (<0.006 ng/ml of endotoxin; Irvine Scientific, Santa Ana, CA), gentamicin, penicillin, streptomycin, and glutamine. Cells ($0.5\text{--}1 \times 10^7$) were incubated in 35-mm polystyrene petri dishes (Costar, Cambridge, MA) at 37°C and 5% CO₂ in a humidified incubator for 1.5–2.5 h. After this time, nonadherent cells were washed off and fresh medium was added to the dishes. Adherent cells were >90% esterase-positive and <1% CD3-positive.

Culture Conditions. Cells were cultured in the presence of various concentrations of the different cytokines at 37°C and 5% CO₂ for 48 h unless otherwise stated. The dishes were then placed at 4°C for 30 min and gently scraped with a rubber policeman to remove adherent cells. Viability at this time was ≥95% (trypan blue dye exclusion). Harvested cells were prepared for staining with mAbs (see below). Cytokine-treated cells were compared with cells cultivated in medium alone.

Reagents. Recombinant hGM-CSF (specific activity 10^8 U/mg, LPS content of the concentrated stock <0.025 ng/ml, purity >95%) and hIFN- γ (specific activity 2×10^7 IRU/mg, LPS <0.048 ng/ml, purity >98%) were provided by Amgen Biologicals (Thousand Oaks, CA). Recombinant hIL-1- β (specific activity 5×10^8 U/mg, LPS <0.005 ng/ml, purity >95%) was purchased from the same company. Recombinant hIL-3 (specific activity 10^8 CFU/mg, LPS <0.1 ng/ml, purity >95%) was purchased from Genzyme (Cambridge, MA). Recombinant hIL-4 (specific activity 10^8 U/mg, LPS <0.5 ng/ml, purity >95%) was obtained from Immunex Corp. (Seattle, WA). Recombinant hIL-6 (specific activity 5×10^9 U/mg, LPS <0.006 ng/ml, purity >95%) was a gift from Dr. Martin Lotz (Scripps Clinic Research Foundation, La Jolla, CA). Recombinant hIL-2 (specific activity 3×10^6 U/mg, LPS <0.012 ng/ml, purity >95%) was obtained from Cetus Corp. (Emeryville, CA) and hCSF-1 (specific activity 7×10^7 U/mg, LPS <0.1 ng/ml, purity >95%) was a gift from Dr. R. Halenbeck, Cetus Corp. Recombinant hTNF- α (specific activity 5×10^7 U/mg, LPS <0.008 ng/ml, purity >95%) was provided by Genentech (South San Francisco, CA). In each case, the final concentration of LPS in culture after dilution of recombinant cytokine was <0.005 ng/ml. Polymyxin B (7.900 U/mg) was purchased from Sigma Chemical Co. (St. Louis, MO).

Antibodies. HLA-DR was identified with the mAb I2 (IgG2a, ascites fluid purified by ion exchange chromatography; Coulter Immunology, Hialeah, FL). Leu-10 (IgG1, Becton Dickinson & Co., Mountain View, CA) was used to detect HLA-DQ. CD14 is a monocyte differentiation antigen detected with Mo2 (IgM, ascites fluid purified by ion exchange chromatography; Coulter Immunology) (10). Pooled mouse IgG (Cappel Laboratories, Malvern, PA) or monoclonal IgM (Bionetics, Kensington, MD) were used as controls for most immunofluorescence studies. In some experiments, isotype-matched mouse IgG2a and IgG1 (Coulter Immunology) were used as controls and gave results identical to the pooled IgG.

Immunofluorescence. Cells were stained with mAbs and secondary reagents (FITC-conjugated goat anti-mouse IgG/IgM antibody; Tago, Burlingame, CA), as previously described (11). In some experiments, biotinylated Mo2 and avidin-conjugated FITC (E-Y Laboratories, San Mateo, CA) were used. The results were measured by flow cytometry (see below).

Synovial Tissue Supernatants. Supernatants were prepared from enzymatically dispersed synovial tissue specimens obtained from patients with longstanding seropositive RA as previously described (3). Briefly, the tissue was minced and incubated at 37°C in RPMI 1640 supplemented with 1 mg/ml collagenase (CooperBiomedical, Inc., Malvern, PA) and 50 μ g/ml deoxyribonuclease (Sigma Chemical Co.). The cells were filtered, washed extensively, and cultured in plastic petri dishes in RPMI 1640 plus 10% FCS at a density of $2\text{--}3 \times 10^6$ /ml at 37°C in 5% CO₂. Supernatants were harvested after 72 h, filtered and frozen at –70°C until used.

These supernatants were tested for class II antigen-inducing activity at a 1:5 dilution under the conditions described above. In some experiments the supernatants were incubated for 1 h at room temperature with monoclonal anti-GM-CSF (12) (affinity-purified ascites fluid;

kindly provided by Dr. Ken Kaushansky, University of Washington, Seattle, WA) or anti-IFN- γ (Chemicon, El Segundo, CA) antibodies before their addition to the monocyte cultures.

Flow Cytometry. FACS was performed on a cytofluorograf IIs (Ortho Diagnostics, Raritan, NJ) as previously described (11). 5×10^3 cells in a monocyte-enriched region, as determined by forward and 90° light scatter (containing 90–95% nonspecific esterase-positive cells and 75–80% Mo2⁺ cells) were analyzed from each sample. The fluorescence gain was adjusted in every experiment so that ~5% of cells were positive in the highest fluorescence channel of the sample with greatest fluorescence intensity. All data were displayed on a linear scale of increasing fluorescence without specific units. The results are presented as absolute or relative mean fluorescence channel (MFC) of total cells, the latter being obtained by dividing the absolute MFC of a sample by that of control cells that had been in culture without cytokine for an identical period of time. Forward light scatter was used to estimate the relative cell volume.

Northern Blot Analysis. Total cytoplasmic RNA was isolated from adherent peripheral blood cells using the acid guanidine phenol chloroform method (13). 20 μ g of total RNA was denatured and run on a formaldehyde containing 1.2% agarose gel and blotted onto a nylon membrane (Schleicher & Schuell, Inc., Keene, NH). The HLA-DR β chain probe was obtained from Dr. Per Peterson (Scripps Clinic Research Foundation) and the actin probe was a gift from Dr. Raymond Taetle (San Diego, CA). Probes were radiolabeled with [³²P]dCTP (Amersham Corp., Arlington Heights, IL) by the random primer method (Bethesda Research Laboratories, Gaithersburg, MD). The nylon filter was simultaneously hybridized to both probes overnight at 42°C and washed twice on 2 \times SSC and 0.1% SDS followed by one wash in 0.5 \times SSC and 0.1% SDS at room temperature. The filter was exposed with Kodak X-AR film and an intensifying screen at -20°C for 3 d.

Statistical Analysis. Statistical analysis was performed by two-tailed Student's *t*-test. Data are presented as mean \pm SEM.

Results

Effect of Cytokines on Monocyte HLA-DR Expression. Normal human peripheral blood adherent cells (>90% nonspecific esterase-positive) were incubated either in the presence of medium or medium plus recombinant cytokine. After 48 h, HLA-DR expression was determined by FACS analysis. Table I summarizes the results of these experiments. The concentration of each cytokine was chosen based on near-maximal activity in standard biological assays (e.g., CTLL proliferation for IL-2). More extensive dose responses were performed with TNF- α (1–500 ng/ml), CSF-1 (10–1,000 U/ml), IL-4 (5–100 U/ml), and IFN- γ (1–500 U/ml). The concentration with the

TABLE I
Effect of Cytokines on Monocyte HLA-DR Expression

Cytokine	n	Concentration	Relative MFC*
GM-CSF	16	800 U/ml	2.54 \pm 0.33 (<i>p</i> < 0.001)
IFN- γ	16	100 U/ml	5.14 \pm 0.60 (<i>p</i> < 0.001)
TNF- α	9	40 ng/ml	1.31 \pm 0.06 (<i>p</i> < 0.02)
IL-4	4	50 U/ml	1.20 \pm 0.03 (<i>p</i> < 0.01)
IL-1 β	3	40 U/ml	1.05 \pm 0.04
IL-2	4	10 ng/ml	1.01 \pm 0.06
IL-3	2	50 U/ml	0.97
IL-6	3	100 U/ml	1.01 \pm 0.06
CSF-1	5	1000 U/ml	1.03 \pm 0.05

Data presented as mean \pm SEM. *p* > 0.1 unless otherwise indicated.

* Relative MFC = MFC cytokine/MFC medium.

maximal activity was used for these cytokines (see Table I). CSF-1 (M-CSF), IL-1, IL-2, IL-3, and IL-6 had no effect on HLA-DR expression. TNF- α increased surface HLA-DR ($p < 0.02$), but the effect was small. Similarly, IL-4 induced a modest increase in monocyte Ia expression ($p < 0.01$).

As expected, IFN- γ was the most powerful inducer of HLA-DR, increasing expression to 5.14 ± 0.60 of control monocytes ($p < 0.001$). The only other cytokine that increased Ia at least twice control was GM-CSF (relative MFC = 2.54 ± 0.33 , $p < 0.001$). Fig. 1 shows representative histograms of GM-CSF and IFN- γ mediated HLA-DR induction. To rule out the possibility that the effect of GM-CSF was modified by contaminating LPS, similar experiments were performed in the presence and absence of polymyxin B (16 $\mu\text{g/ml}$). No significant difference was observed between the two conditions (data not shown).

Using FACS analysis, relative cell volume was estimated using forward light scatter for GM-CSF-treated monocytes and control monocytes and there was no significant difference (data not shown). Therefore, the increase in MFC in GM-CSF cultures was due to a change in antigen density rather than simply due to an increase in cell size.

Characterization of Surface Antigen Modulation by GM-CSF. Fig. 2 shows a representative dose response for the induction of HLA-DR by GM-CSF. An effect was generally observed with 25 U/ml and was maximal with 80–800 U/ml. In kinetics experiments,

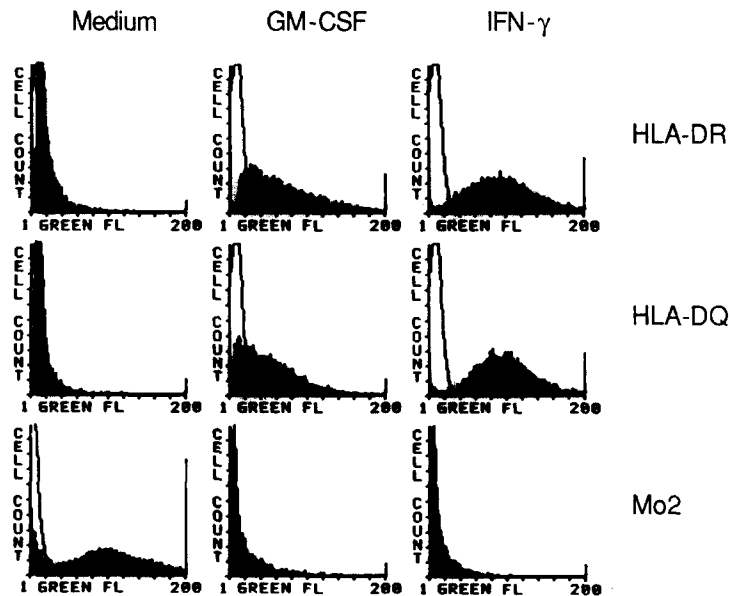


FIGURE 1. Representative histograms of the effect of GM-CSF and IFN- γ on HLA-DR, HLA-DQ, and Mo2 expression by monocytes. Vertical axis of each histogram represents relative cell number and horizontal axis represents relative fluorescence. In the first column (i.e., cells cultured in medium alone), the shaded histogram represents cells stained with the indicated mAb and the open histogram shows the cells stained with the control antibody. In the GM-CSF and IFN- γ columns, both the shaded histograms (cytokine-treated cells) and the open histograms (cells cultured in medium) represent cells stained as indicated on the right hand side of the figure.

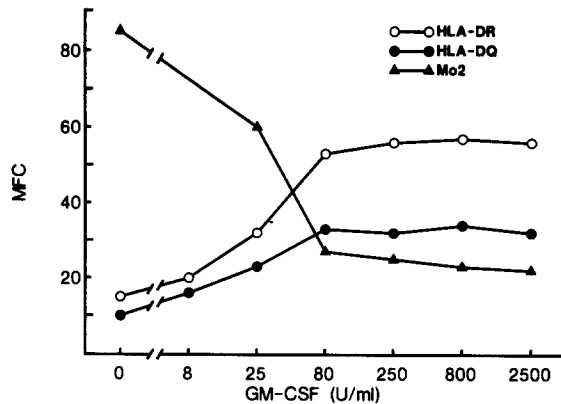


FIGURE 2. Dose responses of the effect of GM-CSF on the expression of HLA-DR, HLA-DQ, and Mo2 on human monocytes. Cells were incubated for 48 h in the presence of different concentrations of GM-CSF. Results are expressed as absolute mean fluorescence channel (MFC).

an increase in DR expression was detected within 24 h and was usually maximum after 48 h (data not shown).

GM-CSF also modulated the surface expression of other monocyte antigens (Figs. 1 and 2). For instance, GM-CSF induced the expression of HLA-DQ on human monocytes. The cytokine was also a potent downregulator of CD14 (a monocyte/macrophage-specific surface protein detected by the mAb Mo2). Surface expression of CD14 is also decreased by IFN- γ (11).

HLA-DR Induction by GM-CSF Is Not Mediated by IFN- γ . Because the effect of GM-CSF on HLA-DR, HLA-DQ, and Mo2 paralleled that of IFN- γ , we considered whether the GM-CSF acted through the induction of IFN- γ production by contaminating T cells in vitro. This was felt to be unlikely because the cells studied contained <1% CD3⁺ cells. Furthermore, IL-2, which is known to induce T cell IFN- γ production (14, 15), had no effect on Ia expression. Finally, further T cell depletion by SRBC rosetting before plastic adherence did not interfere with GM-CSF-mediated HLA-DR induction (data not shown). To address this possibility directly, monocytes were incubated with GM-CSF in the presence of a neutralizing mAb to IFN- γ . The antibody alone had no effect on HLA-DR expression. Table II shows that anti-IFN-gamma antibody neutralized 77% of IFN- γ mediated HLA-DR induction, but had no effect on GM-CSF-mediated HLA-DR expression.

Interactions Between GM-CSF and Other Cytokines. The effect of GM-CSF on HLA-DR expression was additive with low levels of IFN- γ (see Table III for a representa-

TABLE II
Effect of GM-CSF Is Not Mediated through IFN- γ

	Alone	Plus anti-IFN- γ
Medium	18*	18
GM-CSF (800 U/ml)	31	31
IFN- γ (10 U/ml)	91	35

Samples were preincubated with or without anti-IFN- γ antibody for 1 h before addition to monocytes. Cells were harvested after 48 h. Standard error is <2 channel in each case.

* Data presented as MFC.

TABLE III
Interaction Between GM-CSF and IFN- γ , TNF- α , and IL-4*

GM-CSF and IFN- γ	MFC [†]
Medium	16
GM-CSF (800 U/ml)	29
IFN- γ (1 U/ml)	25
GM-CSF (800 U/ml) + IFN- γ (1 U/ml)	35
IFN- γ (100 U/ml)	65
GM-CSF (800 U/ml) + IFN- γ (100 U/ml)	64
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GM-CSF and TNF- α	
Medium	15
GM-CSF (800 U/ml)	36
TNF- α (40 ng/ml)	17
GM-CSF (800 U/ml) + TNF- α (40 ng/ml)	53
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GM-CSF and IL-4	
Medium	26
GM-CSF (800 U/ml)	43
IL-4 (50 U/ml)	35
GM-CSF (800 U/ml) + IL-4 (50 U/ml)	48

* Monocytes were cultivated for 2 d with either medium or the combination of cytokines indicated. Similar results were seen in two to four separate experiments.

[†] MFC, mean fluorescence channel in linear scale. Standard error was <2 channel in each case.

tive experiment). In the presence of high concentrations of IFN- γ , however, GM-CSF had no additional effect. In contrast, TNF- α , which slightly increased HLA-DR expression on normal human monocytes, synergized with GM-CSF in the induction of DR. IL-4 had a slightly additive effect with GM-CSF (see Table III). CSF-1, IL-1, and IL-2 had no effect on GM-CSF-mediated modulation of Ia antigens (data not shown).

GM-CSF Increases HLA-DR mRNA Levels. To determine whether GM-CSF increased expression of class II MHC genes, cytoplasmic RNA was prepared from adherent monocytes and mRNA was analyzed by Northern blot analysis. HLA-DR mRNA levels from monocytes cultured either in medium or GM-CSF for 2 d was determined. HLA-DR mRNA content was higher in the GM-CSF-treated monocytes compared with control monocytes (see Fig. 3).

The HLA-DR-inducing Factor in Synovial Tissue (ST) Explant Supernatants is Neutralized by Anti-GM-CSF Antibody. High levels of surface HLA-DR expression in the synovial lining is a cardinal finding in RA (1). Culture supernatants from synovial tissue cells contain a factor that induces HLA-DR expression on normal monocytes and is not neutralized by anti-IFN- γ antibody (3). Because we have recently shown that GM-CSF is produced locally by the rheumatoid synovium and is present in RA synovial effusions (5), we tested the ability of a specific mAb to GM-CSF to neutralize the non-IFN- γ Ia-inducing activity in joint samples. Culture supernatants from four rheumatoid synovial tissue explants were incubated with normal monocytes in the presence or absence of anti-GM-CSF antibody for 48 h. In each case,

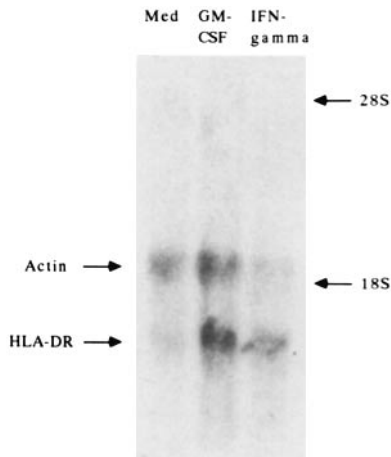


FIGURE 3. Induction of HLA-DR β chain mRNA by GM-CSF and IFN- γ . Monocytes were cultured in medium, 800 U/ml GM-CSF, or 100 U/ml IFN- γ for 2 d. Approximately 20 μ g/ml of cytoplasmic RNA was loaded in each lane. Actin and HLA-DR probes were simultaneously hybridized to the nylon membrane. The density of the actin band was proportional to the total RNA content as judged by ethidium bromide staining.

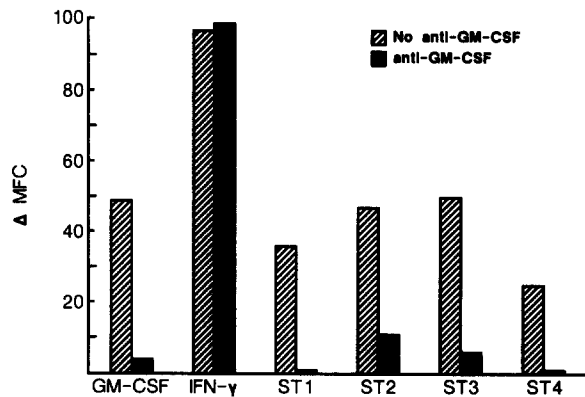


FIGURE 4. Effect of anti-GM-CSF mAb on synovial tissue explant supernatant-mediated HLA-DR induction. Samples were preincubated with anti-GM-CSF antibody (350 U/ml of neutralizing activity) for 1 h at room temperature before addition to cultures. After 48 h, cells were harvested and stained. Results are expressed as Δ MFC (mean fluorescence channel of test cells minus the MFC of control cells cultured in the presence of medium alone).

the antibody prevented induction of HLA-DR (see Fig. 4) (Δ MFC_{ST} = 39.5 ± 5.7 ; Δ MFC_{ST+anti-GM-CSF} = 4.7 ± 2.4 ; $p < 0.01$). The antibody did not interfere with IFN- γ -mediated HLA-DR induction. Anti-IFN- γ antibody did not neutralize the factor in synovial tissue supernatants (data not shown).

Discussion

This study represents a comprehensive examination of the Ia-inducing capacity of a number of cytokines on normal human monocytes. Of particular interest is the observation that GM-CSF induced high levels of surface HLA-DR and HLA-DQ expression. The observation that GM-CSF induces Ia expression on human cells of monocyte/macrophage lineage has precedence in murine systems. For instance, GM-CSF increases class II antigen expression on murine peritoneal macrophages (6). Also, GM-CSF-derived bone marrow macrophages express more Ia than M-

CSF derived cells in mice (7). However, there are no studies describing the effect of GM-CSF on Ia expression on human cells. In addition to its effect on Ia density, GM-CSF also downregulated CD14 on monocytes. Low CD14 expression, particularly in association with high levels of surface HLA-DR, has been described as an activation phenotype for human monocytes and macrophages (11). This phenotype has been noted in inflammatory synovial fluids (16), as well as alveolar macrophages from patients with sarcoidosis (17).

Quantitative variation in Ia expression plays a major role in immune regulation (18). The ability of GM-CSF to modulate HLA-DR and DQ may have important physiological implications in the normal immune response. For example, parenteral administration of GM-CSF to mice augments the primary antibody response (6). This effect is due to enhanced function of APCs, including increased Ia expression (6, 9). The fact that monocytes and macrophages obtained from inflammatory foci resemble GM-CSF-treated monocytes suggests that local production of GM-CSF (either with or without IFN- γ) could play a role in macrophage activation in disease states.

As a rule, cytokines are noted for highly redundant activities, but the ability to induce HLA-DR expression was limited to only a few. GM-CSF and IFN- γ were by far the most powerful mediators. The increase in surface HLA-DR expression after GM-CSF exposure corresponded to an increase in HLA-DR mRNA levels, which suggests that the mechanism was pretranslational. TNF- α , which reportedly induces Ia on murine macrophage cell lines (19) and HLA-DR on some human tumor cells (20), had a limited (albeit statistically significant) activity. More striking, however, was its ability to synergize with GM-CSF. Previous reports have suggested that IL-4 is an Ia inducer on human monocytes (21, 22) and murine macrophages (23, 24). Although our data confirm this, the degree of activity was much less than that found for either IFN- γ or GM-CSF. CSF-1 (M-CSF) has been reported to increase Ia expression on human monocytes cultured in serum-free medium (25). However, in our system, none of the other factors tested (CSF-1, IL-1, IL-2, IL-3, IL-6) significantly altered monocyte Ia expression either alone or in combination with GM-CSF.

One of the most consistent findings in the synovium of patients with RA is the large amount of Ia antigens detected on the macrophage-like type A synovial lining cells and synovial tissue macrophages (1). Because these cells are derived from the bone marrow (9) and are likely either related to or derived from monocytes in the peripheral blood, monocytes are a convenient model for studying synovial macrophage HLA-DR expression. The precise site where cells receive the signal is not known, although it is often presumed to be intra-articular. The identification of Ia-inducing factors in the joint would support this. In previous investigations of the mechanism of Ia induction on macrophages in the joint we have demonstrated that: (a) low levels of IFN- γ are present in the joint in RA (3); (b) monocytes from patients with RA are not uniquely sensitive to the effects of low levels of IFN- γ (4); and (c) a non IFN- γ Ia-inducing factor is present in rheumatoid synovial fluid and culture supernatants of RA synovial cells (3). Two lines of evidence support the notion that GM-CSF is an Ia-inducing factor in RA. First, using bioassays and a specific RIA, significant levels of GM-CSF have been identified in RA joint samples (5). Hence, this protein is present in sufficient quantities in the joint (40–140 U/ml in synovial tissue supernatants) to account for the activated phenotypes (i.e., high Ia and low

CD14) (16) observed. Second, the Ia-inducing activity in RA synovial tissue supernatants was blocked by antibody to GM-CSF, but not by antibody to IFN- γ .

Although GM-CSF is likely an important Ia-inducing factor in the joint, other cytokines are probably also involved. For instance, low levels of IFN- γ (similar to those found in synovial fluid) have an additive effect on GM-CSF-mediated induction of HLA-DR expression. TNF- α , which has also been reported in RA synovial fluid (26), synergizes with GM-CSF. Therefore, the induction and maintenance of the large amounts of class II molecules on the surface of rheumatoid macrophages and synoviocytes is likely due to complex interactions of GM-CSF, TNF- α , low levels of IFN- γ and possibly other cytokines. Studies to identify the cells responsible for secretion of these cytokines are currently in progress.

Summary

Granulocyte/macrophage CSF (GM-CSF) has recently been identified in rheumatoid arthritis (RA) synovial effusions. To study a potential role for GM-CSF and other cytokines on the induction of HLA-DR expression on monocytes and synovial macrophages, we analyzed the relative ability of recombinant human cytokines to induce the surface expression of class II MHC antigens on normal peripheral blood monocytes by FACS analysis. GM-CSF (800 U/ml) (mean fluorescence channel 2.54 ± 0.33 times the control, $p < 0.001$) and IFN- γ (100 U/ml) (5.14 ± 0.60 , $p < 0.001$) were the most potent inducers of HLA-DR. TNF- α and IL-4 also increased HLA-DR expression, although to a lesser degree [1.31 ± 0.06 ($p < 0.02$) and 1.20 ± 0.03 ($p < 0.01$), respectively]. IL-1 (40 U/ml), IL-2 (10 ng/ml), IL-3 (50 U/ml), IL-6 (100 U/ml), and CSF-1 (1,000 U/ml) did not affect surface HLA-DR density. GM-CSF also increased HLA-DR mRNA expression and surface HLA-DQ expression, but decreased CD14 (a monocyte/macrophage antigen) expression. The effect of GM-CSF on HLA-DR was not mediated by the generation of IFN- γ in vitro because it was not blocked by anti-IFN- γ mAb. GM-CSF was additive with IL-4 and low amounts (<3 U/ml) of IFN- γ and synergistic with TNF- α . Because we have recently reported that supernatants of cultured RA synovial cells produce a non-IFN- γ factor that induces HLA-DR on monocytes, we then attempted to neutralize this factor with specific anti-GM-CSF mAb. Four separate synovial tissue supernatants were studied, and the antibody neutralized the HLA-DR-inducing factor in each ($p < 0.01$).

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