Mechanisms of Tumor Necrosis Factor-Granulocyte-Macrophage Colony-Stimulating Factor-Induced Dendritic Cell Development

By Frances Santiago-Schwarz, Nicholas Divaris, Corinne Kay, and Steven E. Carsons

In a previous report, we described that tumor necrosis factor (TNF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) synergistically enhanced the development of dendritic cell (DC) progeny from early stem cells and that there is a common monocyte-DC progenitor cell. Low levels of DC were obtained with GM-CSF alone, and TNF by itself failed to induce stem cell development. Here, we investigate mechanisms by which TNF and GM-CSF induce increases in DC, and how these same molecules support later stages of DC differentiation. We show that TNF is required as the first signal, that there is upregulation of GM-CSF receptors (GM-CSFRs), and that TNF inhibits the differentiation of colony-forming units—granulocyte. High levels of GM-CSFRs were always associated with conditions yielding a large number of DC, and a kinetic analysis showed a close ontogenic relationship between DC and GM-CSFR levels. The addition of anti–GM-CSF or anti-TNF antibodies blocked synergistic responses related to DC development, including high levels of GM-CSFRs. Anti–GM-CSF was the most potent inhibitor of proliferation (80%) and macrophage, DC, and polymorphonuclear (PMN) cell development. With polyclonal anti-TNF, inhibition was less (35%), and there was a shift from myelomonocytic and DC to PMN progeny. Our results support the concept that receptor upregulation is an important mechanism for growth factor synergy. Our data also indicate that the opposing effects of TNF on hematopoiesis contribute to the selection of the DC pathway and emphasize the importance of GM-CSFRs not only in initiating DC development, but also in controlling DC viability and function. © 1993 by The American Society of Hematology.

The development of differentiated progeny from hematopoietic stem cells is a complex multistep process involving the positive and negative regulatory actions of cytokines. In the erythroid and myeloid lineages, the differentiation of common progenitors may be initiated by nonlineage restricted cytokines such as interleukin-1 (IL-1), IL-3, and stem cell factor (SCF). Ultimate development and enhancement of mature erythroid, monocytic, and granulocytic progeny occurs in the presence of erythropoietin, monocyte colony-stimulating factor (M-CSF), and granulocyte colony-stimulating factor (G-CSF), respectively, which are lineage-restricted (late acting) cytokines. The development of restricted progeny can also be achieved by the appropriate combination of multilineage cytokines (granulocyte-macrophage colony-stimulating factor [GM-CSF], IL-3) with other regulatory proteins. In these cases, enhanced colony formation is not supported by either growth factor alone. The development of eosinophils with GM-CSF and IL-3, and of basophils with GM-CSF, IL-3, and IL-4 are specific examples of these synergistic interactions.

We and others have recently shown that the dendritic cell pathway can be enhanced by the synergistic actions of two pleiotropic cytokines. We noted that purified CD34+ stem cells from neonatal cord blood treated with tumor necrosis factor (TNF) and GM-CSF underwent proliferative responses and matured into cells exhibiting the morphologic and functional characteristics of dendritic cells. The simultaneous development of macrophages also occurred in these cultures. The presence of colonies containing both macrophages and dendritic cells (DCs) showed a common monocyte-DC progenitor and the close ontogenic relationship between these two cell types. GM-CSF was the only cytokine tested that by itself, induced low levels of DCs, and TNF in the absence of GM-CSF was incapable of inducing stem cell development.

Investigators studying the capacity of TNF to enhance GM-CSF–induced proliferation of AML cells determined that TNF promoted upregulation of GM-CSF receptors (GM-CSFRs) and suggested this as a mechanism for TNF and GM-CSF synergy. Other reports on the potentiation effects of TNF on the monocytic pathway have indicated that downregulation of the colony-forming unit-granulocyte (CFU-G) pathway leads to subsequent enhancement of the colony-forming unit-monocyte (CFU-M) pathway. In this study, we investigate whether TNF and GM-CSF—amplified DC development involves either of these mechanisms. Our results support that, in the least, both these mechanisms are involved. In addition, we establish that GM-CSFRs are not only upregulated during early phases of DC development but are maintained at high levels during the entire DC pathway. Finally, the effects of antibodies to GM-CSF and TNF in blocking DC development are discussed.

Materials and Methods

Enrichment of neonatal cord blood-derived stem cells. Neonatal cord blood was collected into sterile heparinized containers from healthy full-term infants, according to institutional guidelines, at the time of repeat caesarean sections. The blood was diluted and layered over a pyrogen-poor Lymphoprep (Nyegaard, Norway) gradient for the isolation of mononuclear cells (MNCs). MNCs were then placed on two consecutive nylon wool columns (NWXZ) for the enrichment of nonadherent stem cells as previously described. This procedure results in fourfold to fivefold enrichment of CD34+ cells and the complete removal of mature hematopoietic elements except T and natural killer (NK) cells, as previously shown.

From the Winthrop-University Hospital, Mineola, NY; and SUNY, Stony Brook, NY.

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Address reprint requests to Frances Santiago-Schwarz, PhD, Division of Rheumatology, Immunology and Allergy, 222 Station Plaza, Suite No. 430, Mineola, NY 11501.

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Subsequent purification of CD34+ cells used either antibody and complement (C) depletion of T and NK cells, or positive selection using immunomagnetic beads. For antibody and C depletion, NWWx2 nonadherent cells were incubated with monoclonal antibodies (MoAbs) to T-cell (CD3, OKT3) and NK-cell (CD57) antigens (American Type Culture Collection, Rockville, MD) for 40 minutes at 4°C, washed twice in RPMI 1640, incubated with rabbit C (1:15) for 45 minutes at 37°C, and then washed twice again. This procedure results in a cell population (NWX2-T, -NK) with a viability of greater than 90% and containing ~20% CD34+ cells. Controls consisted of C treatment in the absence of MoAbs. For positive selection, NWx2 cells were incubated with MoAb to CD34 (Becton Dickinson, Mountain View, CA) for 40 minutes at 4°C, washed twice, and then reacted with antimouse IgG linked to immunomagnetic beads (Dynabeads; Dynal Inc, Great Neck, NY) to a bead to cell ratio of 5:1. After 50 minutes, cells expressing CD34 antigens were positively selected using a magnetic cell concentrator (Dynal MPC-1). The beads were removed from the cells using Detachabead (Dynal), as suggested. The final cell population after this treatment consisted of cells that were greater than 90% CD34+ and greater than 95% viable, as determined by trypan blue exclusion.

Culture conditions for DC growth. CD34+-enriched cell populations and pure CD34+ cells were adjusted to 0.5 to 1.0 x 10^6 cells/mL, respectively, in RPMI 1640 medium containing 2 mmol/L L-glutamine, 10 mmol/L HEPES, 50 IU/mL penicillin, 50 μg/mL streptomycin, and 5% pooled normal human serum (NHS/RPMI). This concentration of NHS does not sustain stem cell proliferation, as previously shown. Recombinant TNF-α (Koll Pharmaceutical, Whippany, NJ) was added to stem cell populations in NHS/RPMI at 500 U/mL; recombinant GM-CSF (Genzyme, Boston, MA) was added at 100 U/mL. The optimal concentration for each factor was determined as previously described. Cells were incubated at 37°C in a 5% CO2 humidified incubator in either Teflon culture vials (Scientific Specialties Service, Randalstown, MD) or 24-well tissue culture plates. Cells cultured in suspension (Teflon), when transferred to adherent conditions at the onset of the culture period. The endotoxin levels were controlled, as previously described.

In situ analysis showed that cells exhibiting dendritic cell morphology were positive for class II major histocompatibility complex (MHC) antigens but CD14+, did not exhibit nonspecific esterase activity, and were incapable of phagocytosing latex bead particles, as previously described.

Sequential studies. To assess whether TNF or GM-CSF was required as a first signal in TNF + GM-CSF synergy, cells were preincubated with either recombinant TNF-α (Koll Pharmaceuticals) at 500 U/mL or recombinant GM-CSF (Genzyme) at 100 U/mL for 1, 3, 5, and 7 days. At each time point, cells were removed from culture, washed to remove any endogenous cytokines, and the second cytokine was added. After further incubation, proliferative and morphologic analyses were performed.

Proliferative capacity of cultured cells. Cells were analyzed for proliferation by the uptake of [3H] thymidine and by manual cell counts using a hemacytometer (Improved Neubauer; Fischer Scientific, Pittsburgh, PA). For thymidine uptake, 0.5 μCi of [3H] thymidine (specific activity, 25 Ci/mmol/L; Amersham, Arlington, IL) was added to 100 μL of cells in 96-well microtiter plates. After 4 hours of incubation, cells were harvested using an automated sample harvester and counted in a liquid scintillation counter. Results are expressed as the mean of triplicate counts; the standard error was ≤15% in all experiments.

Blocking antibodies. The effect of antibodies to TNF and GM-CSF in blocking DC development was investigated by adding one of these reagents to stem cell populations containing TNF and GM-CSF at the onset of the culture period. Mouse antihuman GM-CSF MoAb antibody (Genzyme) was tested at concentrations of 10 and 20 μg/mL. Polyclonal antihuman TNF-α (Genzyme) was tested at doses of 5, 10, and 25 μg/mL. MoAb antihuman TNF-α (UBI, Lake Placid, NY) was analyzed at 50, 100, and 500 ng/mL. Both antibodies were functionally defined in bioassays, and were effective at neutralizing TNF-mediated cytotoxicity, but no studies on the effects of TNF regulation of hematopoiesis were reported. The MoAb did not block the proliferative capacity of the stem cells or their pattern of differentiation, whereas the polyclonal did. This suggests that different epitopes on TNF regulate cytotoxic and hematopoietic functions. Nonspecific effects were addressed by adding isotropic control antibodies (IgG, IgM; Coulter Immunology, Hialeah, FL) at saturating concentrations instead of anti-TNF or GM-CSF.

GM-CSFR ligand studies. GM-CSFRs were detected using a GM-CSFR kit purchased from R&D Systems (Minneapolis, MN). This strategy uses the binding of ligand to a fluorochrome to facilitate the detection of cell surface receptors by flow cytometric analysis. In this particular application, cell populations expressing GM-CSFRs are identified and increasing intensity of fluorescence correlates with increasing GM-CSFR levels. Assays were performed according to the manufacturer’s instructions. Briefly, 10 μL (0.1 μg) of biotin-labeled recombinant GM-CSF was added to approximately 1 x 10^6 cells for 1 hour at 4°C. Cells were then washed twice, fixed in 10% buffered formalin for 5 minutes at room temperature, and washed in phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin (BSA), 0.1% sodium azide (PBS/BSA/azide) in preparation for flow cytometry (FACSscan; Becton Dickinson). Calibration of the instrument was performed with Calibrate beads (Becton Dickinson). A total of 10,000 events was analyzed using 488 nm wavelength excitation. Data were analyzed using a LYSYS software program (Becton Dickinson). Debris and dead cells were omitted on the basis of forward and right angle scatter. Controls for nonspecific fluorescence consisted of treating paired samples from each culture condition with avidin-FITC alone.

Receptor blocking studies. Specificity of the biotin-labeled GM-CSF binding to cell surface GM-CSF receptors was established in competition assays as follows: 1.0 x 10^6 CD34+ stem cells were incubated with 5 μg (50-fold molar excess) of human recombinant GM-CSF (R&D Systems) at 4°C with frequent gentle resuspension. After 30 minutes, 0.1 μg of biotin-conjugated GM-CSF was added to the tube, the suspension was mixed, and the GM-CSFR assay was completed as indicated above.

Statistics. Student’s t-test analysis was performed using a Crunch Interactive Statistical Package (CRISP; Crunch Software Corp, San Francisco, CA).

RESULTS

Sequential studies. We sought to determine whether TNF or GM-CSF was the first cytokine required in signaling synergistic dendritic cell development. Figure 1 depicts cells pretreated with TNF for 1 to 7 days before the addition of GM-CSF. Cells cultured with TNF for up to 3 days with subsequent addition of GM-CSF exhibited DC content similar (25% to 40%) to that obtained when GM-CSF and TNF were added simultaneously at day 0 (Fig 1A, B, and D). After day 5, DC content decreased dramatically (Fig 1C), In
Fig 1. Effects of TNF pretreatment on DC development. Stem cells were preincubated with TNF for 1, 3, 5, and 7 days. At each time point, the cells were removed from culture, washed, and fresh media supplemented with GM-CSF was added. (A) 1 day, (B) 3 days, and (C) 7 days of TNF pretreatment. (D) TNF + GM-CSF added simultaneously on day 0. (E) GM-CSF alone on day 0. Photomicrographs were taken after a total of 12 days in culture. One of six typical experiments is depicted. Original magnification × 50.

In fact, the sequential addition of GM-CSF to cells pretreated with TNF for 7 days (Fig 1C) yielded little stem cell development and a population of cells that resembled treatment with TNF alone. The reciprocal experiment, involving pretreatment with GM-CSF followed by the addition of TNF at various periods (1, 3, 5, and 7 days), did not show increases in DC content other than those noted with GM-CSF alone (≤10%) (Fig 1E). There was no stem cell development in the presence of 5% NHS/RPMI alone.

These experiments indicate that TNF, not GM-CSF, is required as the first signal and that there is sensitivity to the secondary effects of GM-CSF for up to 72 hours. Other investigators found that TNF/GM-CSF synergistic effects on CD34+ human bone marrow cells were restricted to the early addition of TNF, in support of these observations.

Proliferative responses associated with TNF or GM-CSF pretreatment. In Fig 2, we compare thymidine uptake in stem cells preincubated with either TNF or GM-CSF for 72 hours followed by the addition of GM-CSF and TNF, respectively. On day 7, marked proliferation was noted in cultures pretreated with TNF but not those pretreated with GM-CSF. In the two experiments shown, increases
over GM-CSF pretreatment were 2.6- to 9.0-fold (P < .04) (Fig 2B and A, respectively). Uptake in NHS controls was ≤300 cpm. Microscopic examination showed DC content similar to that observed with the simultaneous addition of TNF + GM-CSF (Fig 1D). Moreover, the proliferative capacity of the cells pretreated with TNF approximated that of simultaneous addition of TNF and GM-CSF at the onset of culture (Fig 2B). Increases in cell number in the TNF-pretreated cultures were confirmed by manual cell counts (data not shown).

Cells pretreated with GM-CSF followed by TNF exhibited a marked inhibition in proliferation on day 7 (Fig 2). The effect was reversible, as noted by the rebound on day 10 (Fig 2A and B). Microscopic examination, Wright stain analysis, and assessment of nonspecific esterase activity showed that on day 10 most cells were macrophages, with some granulocytes present, and that there were no synergistic increases in DCs. In contrast, similar examination of TNF-pretreated cells on day 10 showed synergistic increases in the number of DCs.

Others have shown that TNF has both positive and negative effects on hematopoiesis, in support of these results.

Effect of anti-TNF and anti-GM-CSF antibodies on DC development. Figure 3A depicts inhibition of TNF + GM-CSF–induced stem cell proliferation (measured by thymidine uptake) when anti–GM-CSF is present at the onset of the culture period (P = .0004). Manual cell counts also showed that these cultures contained approximately one-third as many cells as cultures treated with TNF + GM-CSF (P = .015) (Fig 3B). When analyzed under phase microscopy, cultures containing anti–GM-CSF from the beginning (day 0) were sparse, with few DCs, compared with cultures treated with GM-CSF + TNF alone (Fig 4A). Interestingly, addition of anti–GM-CSF later in culture (day 10) resulted in the abrogation of DC morphology within 48 hours (Fig 4D). These results show that DC development can be blocked either at the progenitor cell level or after differentiation events have been induced by the same agent.

Addition of polyclonal anti-TNF interfered with the proliferative capacity of TNF + GM-CSF–treated cells (P = .0025), albeit not as intensely as anti–GM-CSF (35% v 80%, respectively; Fig 3). In contrast, MoAb anti-TNF added at the onset of the culture period did not interfere with stem cell proliferation, even when tested at 500 ng/mL. Isotypic controls (nonimmune mouse IgG and IgM) did not impede cell proliferation, even when tested at 500 ng/mL. Isotypic controls (nonimmune mouse IgG and IgM) did not impede cell proliferation, even when tested at 500 ng/mL. Isotypic controls (nonimmune mouse IgG and IgM) did not impede cell proliferation, even when tested at 500 ng/mL. Isotypic controls (nonimmune mouse IgG and IgM) did not impede cell proliferation, even when tested at 500 ng/mL.}

Even though anti–GM-CSF is a potent inhibitor of stem cell proliferation, some progeny developed in the cultures. As indicated in Table 1 and Fig 5B, large MNC and polymorphonuclear cells (PMN) are the main components (58% and 42%, respectively). Treatment with polyclonal anti-TNF resulted in a more selective lineage outcome. Approximately 75% of the progeny in cultures treated with polyclonal antibody at 5 or 10 μg/mL was PMN. Of these 75%, the majority were basophils (Fig 5A), with the remaining 24% being monocytes; very few DCs were present (Table 1).

Expression of GM-CSFRs. Temporal analysis showed early increases and the persistence of GM-CSFRs throughout the culture period when stem cells were treated with TNF + GM-CSF (Fig 6). The combination of TNF + GM-CSF yielded at least threefold increases in GM-CSFR levels by day 2 (NHS v TNF + GM-CSF, P = .007). In contrast, after 2 days of in vitro culture with GM-CSF or TNF, GM-CSFRs were not significantly increased beyond growth in 5% NHS/RPMI (Fig 6). Although on the rise by day 7, there were still no significant increases in GM-CSFR levels between growth in GM-CSF, TNF, or NHS/RPMI (P > .05). Receptor levels in TNF + GM-CSF–versus GM-CSF–treated day-7 cultures were significantly increased (P = .03).

Very high levels of GM-CSFRs were noted around day 10 in TNF + GM-CSF–treated cultures (TNF + GM-CSF v GM-CSF, TNF, NHS, P < .012). Towards the end of the culture period, receptor levels declined, but were still higher in GM-CSF and TNF + GM-CSF than in TNF or NHS cultures (the differences were not statistically significant).

Early GM-CSFR upregulation did not result from GM-CSF treatment alone (Fig 6). The only significant increases in GM-CSFR levels with GM-CSF treatment were noted on day 10 (v NHS cultures, P = .02). At this point in culture, differentiation events are complete and the cultures contain mostly monocyte-macrophages and few DCs.
MECHANISMS OF DENDRITIC CELL DEVELOPMENT

Fig 3. The effects of anti-TNF and anti-GM-CSF antibodies on stem cell proliferation, as measured by thymidine uptake (A) and hemacytometer-assisted cell counts (B) on day 7. Values represent the mean of two to seven experiments for each condition in (A), and 2 to 5 experiments for each condition in (B). (■) TNF + GM-CSF alone, (□) + MoAb anti-TNF, (△) + polyclonal anti-TNF, (●) + MoAb anti-GM-CSF, (◆) + mslgG + mslgM isotypic controls.

in stem cells. Although gates were set on the flow cytometer to exclude lymphocytes from the study, and GM-CSFRs have not been described on lymphocytes.27,28 We also showed that GM-CSFRs are maintained at high levels during the development of DCs from pure CD34+ stem cells treated with TNF + GM-CSF. As noted in stem cell-enriched studies, GM-CSFR levels were at least twofold greater in pure day-9 cultures treated with TNF + GM-CSF than in cultures treated with GM-CSF (5.32 vs 2.77). After 14 days, GM-CSFR levels in TNF + GM-CSF—treated cultures were still high (6.62).

Fluorescence (FL) versus forward scatter (FSC) dot plot

Fig 4. The influence of anti-GM-CSF during distinct periods of DC development. (A) Typical DC content occurring with TNF + GM-CSF addition on day 0. (B) Cultures were treated with TNF + GM-CSF and anti-GM-CSF at 10 μg/ml from the start. (C) Isotypic control antibody was added together with TNF + GM-CSF on day 0. (D) Anti-GM-CSF was added after 10 days of culture with TNF + GM-CSF. The addition of isotypic antibody to day-10 TNF + GM-CSF—treated stem cells had no effect on cell morphology. All photomicrographs were taken on day 14 of culture. Original magnification × 50.
Table 1. Effect of Anti-TNF and Anti-GM-CSF Antibodies on DC Development

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PMN (%)</th>
<th>Large MNC (%)</th>
<th>DC (%)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM-CSF + TNF exptl.</td>
<td>13</td>
<td>87</td>
<td>40-50</td>
<td>None</td>
</tr>
<tr>
<td>+ Anti-GM-CSF 10 µg</td>
<td>42</td>
<td>58</td>
<td>1-4</td>
<td>80</td>
</tr>
<tr>
<td>+ Anti-TNF (PC) 5 µg</td>
<td>76</td>
<td>24</td>
<td>&lt;1</td>
<td>ND</td>
</tr>
<tr>
<td>+ Anti-TNF (PC) 10 µg</td>
<td>74</td>
<td>26</td>
<td>&lt;1</td>
<td>35</td>
</tr>
<tr>
<td>+ Anti-TNF (MoAb) 50 ng</td>
<td>12</td>
<td>88</td>
<td>40-50</td>
<td>1.0</td>
</tr>
<tr>
<td>+ mlgG + mlgM</td>
<td>12</td>
<td>88</td>
<td>40-50</td>
<td>13</td>
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Differential analysis of cord stem cells cultured in TNF + GM-CSF and various antibody sources for 12 days. A minimum of 500 cells were analyzed by Wright stain and classified as PMN or large MNC. PMN include eosinophils, basophils, and neutrophils. DC cells were identified by the criteria outlined in Materials and Methods and quantitated by analyzing day-12 cultures under phase microscopy. The percentage of inhibition refers to inhibition of TNF + GM-CSF-induced proliferation, as calculated from the values depicted in Fig 3A.

Abbreviation: ND, not done.

analysis further showed that the highest levels of GM-CSFRs were not associated with cells exhibiting the greater FSC, but were consistently on intermediate-sized cells, as shown in Fig 7A. This indicates that the highest levels of GM-CSFRs are not exhibited on larger macrophages, consistent with our findings that GM-CSF treatment alone, while producing mostly macrophage progeny, does not elicit highest levels of GM-CSF (Fig 6).

Finally, the effect of anti-TNF and anti-GM-CSF on the expression of GM-CSFR was also investigated. As summarized in Table 2, the levels of receptor correlated closely with the effects of the particular antibody on the DC pathway. Thus, anti–GM-CSF and polyclonal anti-TNF, which severely hampered DC development (Table 1 and Fig 4), also inhibited the expression of GM-CSFR. Conversely, MoAb anti-TNF, which had no effect on the DC pathway, did not interfere with GM-CSFR levels.

Specificity of the GM-CSFR reaction. Competition studies showed the specificity of the reagents used to detect GM-CSF receptors. Pretreatment of TNF + GM-CSF cultures (derived from pure CD34+ stem cells) with a 50-fold molar excess of unbound GM-CSF resulted in approximately 40% inhibition of the binding reaction, as shown in Fig 7B.

DISCUSSION

In this report, we provide insight into how TNF and GM-CSF initiate the generation of DCs from early stem cells and how these same molecules are involved in maintaining mature DC progeny. Our studies support the developing con-
Several attempts were made to manipulate the DC pathway with antibodies to TNF or GM-CSF. Anti–GM-CSF was the most potent inhibitor of TNF + GM-CSF synergy. In the presence of this antibody, proliferative events were reduced by ~80% and DC development was greatly affected (Fig 3 and Table 1). There appeared to be no selective inhibition of the granulocytic and monocytic pathways, because the limited progeny arising in the presence of this antibody consisted of similar proportions of PMN and monocyte-macrophages (Table 1). Addition of anti–GM-CSF to mature DC progeny resulted in the abrogation of DC morphology within 48 hours (Fig 4D).

We were able to inhibit TNF + GM-CSF synergy with a polyclonal anti-TNF that involved at least two different mechanisms. One effect was a shift from myelomonocytic to PMN development (Table 1 and Fig 5A). The other effect concerned the DC pathway, as noted by the few DCs present. We speculate that the polyclonal anti-TNF neutralizes the TNF-mediated downregulation of CFU-G, and that the effects on DC development are because of the prevention of TNF binding to cell surface TNF receptors to signal upregulation of GM-CSFR.

The biologic significance of GM-CSFR modulation as a mechanism for DC cell growth regulation is central to this study. Our report upholds that TNF–GM-CSF synergy results in the upregulation of GM-CSFR on stem cells committed to the monocyte-DC pathway during early phases of DC development and that GM-CSF alone does not induce this effect. These results are compatible with reports concerning GM-CSFR levels on AML cells. The highest levels of GM-CSFR were always associated with conditions yielding a large number of DC progeny (TNF + GM-CSF). At the time points tested, TNF and NHS treatment (which does not induce DC development) elicited the lowest levels of GM-CSFR. GM-CSF–treated cultures, which contained mostly monocyte-macrophages and ~10% DC, followed in GM-CSFR content. It is possible that the increased GM-CSFR level on day 10 reflects the DC content in these cultures. However, there were no significant increases in GM-CSFR before day 10, supporting the idea that GM-CSF alone does not upregulate its own receptor and that the increases noted on day 10 may be due to the effects of secondary cytokines.

We previously reported that proliferative responses associated with the development of DC from TNF + GM-CSF–treated CD34+ stem cells increased precipitously after 2 days in culture. Peak proliferation, occurring around days 7 to 10, correlated with peak DC content, and declining proliferative events (~day 14) correlated with loss of DC content. We show here that GM-CSFRs follow the same kinetic pattern, i.e., precipitous increases in GM-CSFR levels occur after day 2, peak GM-CSFR levels are noted around day 10, and declining levels around day 14. We also show that, when development of DCs is impaired with polyclonal anti-TNF or anti–GM-CSF, there is a corresponding decline in GM-CSFRs (Table 2). High levels of GM-CSFRs have been recently described on murine DC and Langerhan cells, as compared with macrophages, lending support to
GM-CSF and TNF both enhance the survival of mature DC in vitro. In addition, these cytokines induce each other's production, especially by monocyte-macrophages. Because DC and monocyte-macrophage development occurs in parallel from a common precursor, we propose that a cytokine loop involving TNF, GM-CSF, DCs, and monocyte-macrophages is a mechanism whereby high levels of GM-CSFRs are maintained during subsequent DC development. Because GM-CSFRs are not usually upregulated by TNF on specialized progeny, this mechanism would be distinctive to the DC lineage. GM-CSF also induces class II MHC expression, which is crucial to the antigen presenting functions of DCs. Other biologic significance of high GM-CSFR levels may include responding to the chemotactic influences of GM-CSF.

Manipulation of the DC pathway such as we describe may be clinically useful in either augmenting DC content in...
immunodeficiency diseases (acquired immunodeficiency syndrome) or decreasing excess DC content in rheumatoid arthritis. Antibodies such as the polyclonal anti-TNF we used in this study would be more effective at specifically targeting DC precursors, while sparing the development of PMN and some monocyte-macrophages. Anti–GM-CSF, which inhibited DC precursors, also had a profound negative effect on PMN and monocyte-macrophages. Because of the negative effects of anti–GM-CSF on mature DCs, it may still be useful for neutralizing the function of differentiated DC progeny.

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Mechanisms of tumor necrosis factor-granulocyte-macrophage colony-stimulating factor-induced dendritic cell development

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