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Human Polymorphonuclear Leukocytes Produce IL-12, TNF-α, and the Chemokines Macrophage-Inflammatory Protein-1α and -1β in Response to Toxoplasma gondii Antigens

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The induction of a type 1 inflammatory cytokine response is a key event in the initiation of immunity to Toxoplasma gondii. Because polymorphonuclear leukocytes rapidly respond to infection by exiting the peripheral blood and accumulating at a site of infection, we sought to determine whether these cells produce cytokines in response to T. gondii. When human peripheral blood neutrophils were stimulated with parasite Ag, they produced both IL-12 and TNF-α. Similarly, up-regulated expression of macrophage-inflammatory protein-1α (MIP-1α) and MIP-1β gene transcripts was induced. Kinetic analysis of IL-12 and TNF-α production revealed distinct patterns following stimulation by T. gondii or LPS. Exogenous TNF-α alone also provided a potent stimulus of MIP-1α and MIP-1β expression, and when neutralizing anti-TNF-α antiserum was included in cultures of parasite-stimulated cells, expression of these CC-family chemokines was partially blocked. These results establish that T. gondii possesses the ability of driving neutrophil proinflammatory cytokine production, and they suggest that parasite-induced MIP-1α and MIP-1β partly results from autocrine stimulation through TNF-α.

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P olymorphonuclear leukocytes (PMN) are well known as one of the earliest cell types arriving at a site of infection. Once there, neutrophils exert anti-microbial activity through their capacity to phagocytose and inactivate pathogens, using mechanisms such as NADPH oxidase-catalyzed production of superoxide and release of neutrophil elastase from azurophil granules (1, 2). During infection, human bone marrow PMN production may increase from 1011 to 1012 per day, although the half-life of these cells in vivo has generally been believed to be on the order of 6–8 h (3). While neutrophils normally undergo rapid apoptosis during culture, several inflammatory cytokines (e.g., IL-1β, TNF-α, IFN-γ, IL-6, G-CSF, GM-CSF) prolong their lifespan, suggesting that PMN may be longer-lived, particularly during infectious settings, than previously believed (4, 5).

In addition to microbicidal activity, PMN are capable of producing several cytokines. Indeed, their ability to rapidly migrate to a focus of infection, as well as their large numbers in peripheral blood, suggest that neutrophils may play an important role as a cytokine source during early infection. In recent years, PMN have been shown to produce both TNF-α and IL-12 after stimulation with microbial products such as bacterial LPS, as well as the yeast pathogen Candida albicans (6–12). Neutrophils are also capable of secreting chemokines of both the CC and CXC families, including the neutrophil chemotactic factor IL-8 and the macrophage chemotactic factors macrophage-inflammatory protein-1α (MIP-1α) and MIP-1β (13–15). Together, these findings suggest that PMN may be important both in early cellular recruitment to a focus of infection and in producing cytokines that influence the activity of the incoming immune cells.

The control of infection with the opportunistic protozoan parasite Toxoplasma gondii is dependent upon strong cell-mediated immunity (16–18). This response is initiated by early IL-12 production from cell types such as macrophages and dendritic cells (19, 20), which can promote macrophage microbicidal function through IFN-γ induction, as well as driving differentiation of Th1 type CD4+ and CD8+ effector T lymphocytes (21). Recently, granulocytes have also been found to contribute to resistance during acute infection. Thus, depletion of PMN with mAb specific for the granulocyte marker GR-1 impairs the ability of mice to survive acute infection with the low-virulence strain ME49 introduced either by i.p. injection or oral administration (22, 23).

The mechanism by which neutrophils contribute to resistance to the parasite is not presently known, but recent work from our laboratory may shed light on this issue. We have found an essential role for PMN in a model of lethal inflammatory cytokine shock induced by the administration of low doses of tachyzoite lysate to β-galactosamine-sensitized mice (24), a result suggesting that neutrophils are involved in Toxoplasma-induced proinflammatory cytokine production in vivo. Nevertheless, this finding does not establish whether PMN themselves release cytokines such as TNF-α and IL-12 in response to T. gondii, or whether neutrophils promote production of these cytokines by another cell type, for example by recruiting and activating monocytes through the release of chemotactic factors (14).

To address this issue, we used human peripheral blood as a source from which to obtain large numbers of purified PMN. As we show in this paper, PMN rapidly release both IL-12 and TNF-α when cultured in vitro with T. gondii Ag. Among several chemokines examined, we found strong up-regulation of transcripts for MIP-1α and MIP-1β after Ag stimulation. The latter CC chemokines were similarly up-regulated by the addition of exogenous...
TNF-α in the absence of further stimulation. These results show that neutrophils are involved in early cytokine responses to the parasite and suggest that they may play a role in establishing the cytokine network initiated by Toxoplasma infection.

Materials and Methods

Parasite extracts

RH strain T. gondii tachyzoites were maintained by biweekly passage on human foreskin fibroblasts in DMEM (Life Technologies, Grand Island, NY), 1% FCS (HyClone, Logan, UT), 100 U/ml of penicillin, and 0.1 mg/ml of streptomycin (Sigma, St. Louis, MO). Soluble tachyzoite Ag (STAg) was prepared as previously described (24). Briefly, tachyzoites were sonicated in the presence of protease inhibitors (0.2 mM PMSF, 0.2 mM aprotinin, 1 mM leupeptin, and 1 mM EDTA), dialoged into PBS, and filtered sterilized through a 0.2-μm membrane (Corning Costar, Cambridge, MA), assayed for protein concentration, and stored at −70°C. Parasite extracts were found to be free of endotoxin as measured by the Limulus amebocyte assay. Parasite cultures were free of Mycoplasma contamination as determined by diagnostic RT-PCR and ELISA (kits from Stragnostec, La Jolla, CA and Boehringer-Mannheim, Indianapolis, IN, respectively), as well as by microbiological assay and fluorescent DNA staining (performed by the Mycoplasma Testing Laboratory, Corell Institute for Medical Research, Camden, NJ)

Toxoplasma ELISA

Peripheral blood from healthy human donors was obtained by venipuncture and collected in vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) containing EDTA. Whole blood was centrifuged (13,000 × g, 5 min). plasma was transferred to a new tube, and an ELISA was performed to test for T. gondii-seropositive individuals as described below.

First, 96-well ELISA plates (Corning Costar) were coated with STAg diluted in PBS (25 μg/ml), and the plates were incubated at 37°C for 2 h. After washing in PBS with 0.05% Tween 20, the plates were blocked by overnight incubation at 4°C in 1% BSA in PBS. Plates were subsequently washed and a serial dilution of each plasma sample was added to wells and incubated at 37°C for 2 h. After washing the plates, a HRP-conjugated goat anti-human Ab (Jackson ImmunoResearch Laboratories, West Grove, PA) was added to sample wells and the plates were incubated a further 90 min at 37°C. After washing, the plates were developed with 2-2-azonio-di(3-ethyl-benzthiazoline sulfonate) (ABTS; Kirkegard and Perry Laboratories, Gaithersburg, MD), absorbance (405 nm) was measured on a Microplate BIO Kinetics Reader (Bio-Tek Instruments, Winooski, VT), and the results were compared with a positive serum pool.

Isolation of human neutrophils

Whole blood was obtained from T. gondii-seronegative individuals by venipuncture and was placed on ice. Neutrophils were isolated as previously described (25) with the following modifications. Before blood collection, an isotonic solution of Percoll (Pharmacia Biotech, Piscataway, NJ) was added to the blood (1:1) for 30 min at room temperature. The band corresponding to the neutrophil fraction was collected by centrifugation at 300 g for 10 min at 4°C. The resulting pellets were washed twice with complete RPMI 1640 media and resuspended in HEPES-buffered RPMI 1640 media with either media alone, LPS (1 μg/ml), or STAg (50 and 100 μg/ml). Tissue culture plates were incubated at 37°C with 5% CO2. Culture supernatants were removed at various times after culture initiation and stored (−70°C) until assayed for the presence of cytokines.

Neutrophil stimulation

PMN were plated in triplicate for each experimental group in a 96-well tissue culture plate (Corning Costar) at 1 × 105 cells/well in complete RPMI 1640 media, with either media alone, LPS (1 μg/ml), or STAg (50 and 100 μg/ml). Tissue culture plates were incubated at 37°C with 5% CO2. Culture supernatants were removed at various times after culture initiation and stored (−70°C) until assayed for the presence of cytokines.

RNA isolation

At the time of supernatant harvest, cells were collected from triplicate wells and pooled in 200 μl of RNA STAT 60 (Tel-Test, Friendswood, TX), then placed in 2 ml Eppendorf tubes. To obtain sufficient RNA for analysis, triplicate samples were pooled. Cellular RNA was subsequently isolated by adding 40 μl of chloroform (Sigma) per 1 × 106 cells, vortexing the tube, and then centrifuging at 13,000 × g for 15 min. The resulting aqueous phase was transferred to a new tube containing an equal volume of iso-propanol (Sigma) and incubated at −20°C overnight. To precipitate RNA, a one-tenth volume of 3 M sodium acetate was added to the RNA-iso-propanol solution, and the mixture was vortexed then centrifuged at 13,000 × g for 15 min. The resulting pellet was washed in 75% ethanol, resuspended in H2O, and the concentration was determined using a spectrophotometer equipped with a UV lamp (Beckman DU-50, Beckman Instruments, Palo Alto, CA).

RT-PCR and Southern blotting

RNA (6 μg) was reverse transcribed using oligo(dT) primers (Promega, Madison, WI). After heating samples to 72°C and chilling on ice to allow hybridization, a master mix (5 × reverse transcriptase buffer, 0.1 M DTT, 2.5 mM dNTPs, 40 U/ml RNasin, and 200 U/ml superscript reverse transcriptase) was added, and the samples were incubated at 45°C for 50 min followed by 10 min at 94°C. The resulting cDNA was either used immediately or stored at −20°C until PCR-mediated gene amplification.

The PCR was performed using a master mix containing dNTPs (2.5 mM), PCR buffer containing 1.5 mM MgCl2 (Promega), primers (0.5 μm; 1/1 anti-sense: sense), and Taq polymerase (5 U/μl; Life Technologies). The nucleotide sequences employed as sense and anti-sense primers were: β-actin, TGAGCAGGCGCTACACCTCGCTGAAGCTCTA; CTAGAAG CATTCCGGGTGAGAGGAGG; MIP-1α, CGGCTGCTGGTCTACCG; MIP-1β, GGCCACCCAGGTGCTGCTTCTTCTCCAG, GTTCGAC GTGTCAGCATCTACTGCTGGACC. The program for PCR consisted of 94°C for 1 min, 54°C for 1 min, and 72°C for 2 min, with a final extension of 7 min at 72°C. The cDNA was amplified 32 cycles (β-actin, 3 cycles (MIP-1α), and 29 cycles (MIP-1β)).

The RT-PCR products were resolved on 2% agarose gels, and bands were visualized by staining with ethidium bromide. A 100-bp DNA ladder (Life Technologies) was simultaneously run on the gels to confirm that PCR products possessed the predicted size. Photographs of gels were scanned and analyzed with the use of Adobe Photoshop software (Adobe Systems, Mountain View, CA). Integrated band size and pixel density was evaluated and expressed as a ratio of chemokine band intensity divided by β-actin band intensity.

Southern blotting was performed as described in detail elsewhere (26). Briefly, amplified DNA was blotted from agarose gels onto a Hybond-N membrane (Amersham International, Buckingham, U.K.) and subsequently probed with internal cytokine-specific oligonucleotides. An enhanced chemiluminescence protocol was employed to detect binding (Amersham). The following probes were employed: MIP-1α, GAGAC CGGCGCTTGGGACTCCTGGCCTG; MIP-1β, AGGAGGTCTG GTCCTCAGTGAAATCTTACA; β-actin, CATGAGGATGATCCTGAC GTGCCGGCCAGC.

Cytokine measurement

Levels of TNF-α and IL-12 (p70) in culture supernatants were measured using commercially available ELISA kits according to the manufacturer’s instructions (Genzyme, Cambridge, MA).

Recombinant cytokine and anti-cytokine treatment

The neutrophils were stimulated in the presence and absence of STAg with recombinant human TNF-α (100 ng/ml) and IFN-γ (20 U/ml) (PharMinGen, San Diego, CA). The induction of MIP-1α and MIP-1β mRNA transcript synthesis was detected by RT-PCR 3 h after culture initiation. For blocking TNF-α, a neutralizing rabbit antiserum (Genzyme) was employed and compared with normal rabbit serum. Both reagents were used at a 1/500 dilution, a 10-fold excess in the amount required to neutralize >95% of the TNF-α activity.

Results

Purification of PMN from T. gondii-seronegative donors

We wished to initially focus our study on donors possessing low T. gondii Ab titers who presumably had not been exposed previously to the parasite. T. gondii-specific serum Ab levels were measured
by ELISA and 4 donors with titers >1/50 were selected for purification of PMN from peripheral blood. As shown in Table I, the populations obtained ranged from 94–99% PMN. The vast majority of these cells were neutrophils, although for the case of donor 1, a relatively large proportion obtained were eosinophils on several separate occasions. Because neutrophils may undergo apoptotic death when cultured for 24 h and beyond (4, 5), we also measured cell viability after 12 h, the time at which our experiments were terminated. As shown in Table I, there was no significant cell death during this period.

T. gondii stimulates neutrophil TNF-α and IL-12 production

Previous studies have shown that human neutrophils are capable of responding to bacterial LPS by producing TNF-α and IL-12 (8, 9), but their ability to produce these cytokines in response to protozoan stimulation is unexplored. Accordingly, PMN cultures were initiated from the seronegative donors listed in Table I, and supernatants were assayed for the presence of IL-12 (p70) and TNF-α after stimulation with media or STAg.

As shown in Fig. 1, parasite Ag induced IL-12 (p70) release from each of the donors. A low level of IL-12 production was found in the absence of parasite stimulation, possibly a result of nonspecific activation during the culture supernatants in response to STAg. Whereas STAg-induced TNF-α reached maximal levels at 6 h and subsequently declined, LPS-stimulated TNF-α production rapidly rose through this period. A similar kinetic profile was found for LPS-driven IL-12 (p70) production, but in contrast to TNF-α, LPS was a less potent stimulus for IL-12 production than was STAg. These and previously published data (24, 27) indicate that the underlying biochemical pathways triggered by LPS and T. gondii, which result in the induction of IL-12 and TNF-α, are distinct.

* PMN were isolated from each individual by centrifugation over a Percoll gradient as described in Materials and Methods.

* Cellular composition was determined visually after staining cytopsin preparations with Diff-Quik.

* Viability was determined by trypan blue exclusion.

Fig. 2 shows the kinetics of cytokine production using PMN from one individual (donor 4). The release of IL-12 (p70) into culture supernatants in response to STAg occurred extremely rapidly, reaching maximal levels within 2 h. We do not at present know if this response is the result of preformed cytokine release, for example by granule exocytosis, or whether it represents the rapid up-regulation of IL-12 gene transcripts. However, similar studies using mouse neutrophils suggest that much of the IL-12 produced in response to STAg results from de novo protein synthesis.5 Interestingly, the kinetics of LPS-driven cytokine production differed from that of STAg (Fig. 2). At 2 h, STAg-induced production of TNF-α was higher relative to LPS. Whereas STAg-induced TNF-α reached maximal levels at 6 h and subsequently declined, LPS-stimulated TNF-α production rapidly rose through this period. A similar kinetic profile was found for LPS-driven IL-12 (p70) production, but in contrast to TNF-α, LPS was a less potent stimulus for IL-12 production than was STAg. These and previously published data (24, 27) indicate that the underlying biochemical pathways triggered by LPS and T. gondii, which result in the induction of IL-12 and TNF-α, are distinct.

FIGURE 1. Human PMN secrete both IL-12 (p70) and TNF-α during in vitro stimulation with T. gondii. Responder cells were obtained from healthy donors with no previous exposure to T. gondii. The PMN were cultured with STAg (ST) or media alone (Med) for 2 h, then supernatants were collected for cytokine measurement. Levels of IL-12 (p70) and TNF-α were measured by cytokine-specific ELISA (see Materials and Methods for details). The results represent mean ± SD values for triplicate wells. In this figure, donors 1–4 correspond to those listed in Table I.

Table I. Donor cell populations after neutrophil enrichment of peripheral blood leukocytes

<table>
<thead>
<tr>
<th>Donor</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
<th>Monocytes</th>
<th>Lymphocytes</th>
<th>Viability at 12 h (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>260.0 ± 18.4 (70%)</td>
<td>88.0 ± 25.6 (24%)</td>
<td>5.0 ± 0 (1%)</td>
<td>17.0 ± 8.5 (5%)</td>
<td>96</td>
</tr>
<tr>
<td>2</td>
<td>231.0 ± 65.1 (92.4%)</td>
<td>6.0 ± 2.8 (2.4%)</td>
<td>4.5 ± 2.1 (1.8%)</td>
<td>8.5 ± 0.7 (3.4%)</td>
<td>93</td>
</tr>
<tr>
<td>3</td>
<td>267.3 ± 32.5 (91.2%)</td>
<td>16.7 ± 3.1 (5.3%)</td>
<td>1.7 ± 1.5 (0.3%)</td>
<td>12.6 ± 2.5 (3.3%)</td>
<td>97</td>
</tr>
<tr>
<td>4</td>
<td>288.1 ± 1.4 (96%)</td>
<td>9.0 ± 1.4 (3%)</td>
<td>0 (0%)</td>
<td>3.0 ± 1.5 (1%)</td>
<td>94</td>
</tr>
</tbody>
</table>

6 S.K.B., Y.Z., and E.Y.D. Mouse neutrophils are a source of high level, IFN-γ-independent IL-12 during Toxoplasma gondii infection. Submitted for publication.
TNF-α initiation.

When exogenous IFN-γ-driven expression of the chemokines appeared slightly augmented, the effect of exogenous IFN-γ possesses the ability to induce MIP-1α and MIP-1β, and up-regulation of these transcripts was sustained for at least 12 h following culture initiation.

The cytokines TNF-α and IFN-γ have been reported to exert effects on cytokine production by PMN. Thus, TNF-α possesses the ability to induce MIP-1α and IL-8, while IFN-γ appears capable of both inhibitory and stimulatory activity dependent upon the time of analysis (32–34). Accordingly, PMN were cultured with STAg or media in the presence of recombinant TNF-α or IFN-γ, and h later cells were harvested and the induction of gene transcripts was examined. Fig. 4, A and B show that exogenous TNF-α alone provided a potent stimulus for increased expression of MIP-1α and MIP-1β gene transcripts. The inclusion of STAg with TNF-α did not appear to further augment the expression of the chemokines. We found a different pattern when examining the effect of exogenous IFN-γ. In this case, the cytokine alone did not induce up-regulation of MIP-1α or MIP-1β transcripts. STAg-driven expression of the chemokines appeared slightly augmented when exogenous IFN-γ was included in the cultures (Fig. 4, A and B).

Because TNF-α alone provided a strong stimulus for MIP-1, we wished to determine whether the expression of this chemokine was driven by TNF-α, itself induced early during STAg stimulation. As shown in Fig. 5, when a neutralizing anti-TNF antiserum was included in the cultures, up-regulation of MIP-1 gene transcription in response to STAg was partially blocked (MIP-1α, 62% inhibition; MIP-1β, 78% inhibition, as estimated from densitometric analysis of scanned gel images). In contrast, normal control serum had no inhibitory effect on STAg-induced chemokine production. We conclude that the T. gondii-induced chemokine response is, in part, driven through TNF-α production. Nevertheless, we cannot exclude the possibility that the parasite directly stimulates neutrophil production of MIP-1α and MIP-1β.

T. gondii induces up-regulation of MIP-1α and MIP-1β in PMN

Because PMN, more than other cell types, are able to rapidly accumulate at a site of infection, we asked whether parasite-stimulated PMN produced chemotactic factors potentially involved in further immune cell recruitment. Both MIP-1α and MIP-1β, members of the CC chemokine family, are chemotactic factors for monocytes and dendritic, NK, and T cells (28–31). As shown in Fig. 3, RT-PCR analysis demonstrates rapid up-regulation of transcripts for both MIP-1α and MIP-1β, and up-regulation of these transcripts was sustained for at least 12 h following culture initiation.

This figure shows the results from a single individual, but similar results were obtained from three additional donors.

FIGURE 2. Kinetics of IL-12 and TNF-α production by PMN in response to T. gondii or LPS. Neutrophils were purified and stimulated with STAg (ST), LPS, or media (Med), and supernatants were harvested for cytokine measurement at the indicated time points after culture initiation. This figure shows the results from a single individual, but similar results were obtained from three additional donors.

The cytokines TNF-α and IFN-γ have been reported to exert effects on cytokine production by PMN. Thus, TNF-α possesses the ability to induce MIP-1α and IL-8, while IFN-γ appears capable of both inhibitory and stimulatory activity dependent upon the time of analysis (32–34). Accordingly, PMN were cultured with STAg or media in the presence of recombinant TNF-α or IFN-γ, and h later cells were harvested and the induction of gene transcripts was examined. Fig. 4, A and B show that exogenous TNF-α alone provided a potent stimulus for increased expression of MIP-1α and MIP-1β gene transcripts. The inclusion of STAg with TNF-α did not appear to further augment the expression of the chemokines. We found a different pattern when examining the effect of exogenous IFN-γ. In this case, the cytokine alone did not induce up-regulation of MIP-1α or MIP-1β transcripts. STAg-driven expression of the chemokines appeared slightly augmented when exogenous IFN-γ was included in the cultures (Fig. 4, A and B).

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FIGURE 3. T. gondii up-regulates neutrophil MIP-1α and MIP-1β gene expression. PMN were purified and incubated with STAg (ST) or media (M). At the times indicated, cells were collected, RNA was isolated, and RT-PCR amplification was conducted as described in Materials and Methods. Amplified PCR products were separated on an agarose gel, stained with ethidium bromide, and photographed (A). A 100-bp ladder (mw, molecular weight) demonstrates that the PCR products possess the predicted size (MIP-1α, 195 bp; MIP-1β, 190 bp; β-actin, 661 bp). B, PCR products were amplified and subjected to Southern blot analysis employing internal chemokine-specific probes. The cell population used in this experiment was comprised of 94.7% neutrophils, 4.6% eosinophils, and 0.7% lymphocytes. Monocytes were not detected. This figure shows results using PMN from one representative donor but similar data were obtained over the course of three additional experiments using other donors.

Discussion

The results of this study demonstrate that human PMN respond to T. gondii stimulation by producing several cytokines, including TNF-α and IL-12. The production of the latter cytokine occurred within 2 h in vitro, and while it is possible that this rapid appearance results from exocytosis of preformed cytokine, RT-PCR analysis indicates that at least some of the IL-12 produced is the result of de novo gene transcription (data not shown). Interestingly, the kinetics of TNF-α and IL-12 production induced by the parasite appear distinct from the response elicited by LPS. Thus, T. gondii was a more potent stimulus for neutrophil IL-12 than was LPS, and conversely, LPS was a much stronger stimulus for TNF-α production than was tachyzoite lysate. These disparate responses suggest that LPS and STAg induced PMN triggering follow distinct molecular pathways, a conclusion that is supported by findings in mouse systems indicating that LPS nonresponsive strains remain responsive to T. gondii-triggered type 1 inflammatory cytokine production (24, 27).

The ability of neutrophils to serve as a cytokine source, in combination with their large numbers in peripheral blood and the ability to rapidly migrate to a focus of infection, suggest that PMN may be a key cell type in cytokine-initiated immune system triggering. The capacity of neutrophils to serve such an immunoregulatory role is underscored by findings in C. albicans-infected mice.
In the latter experimental system, neutrophils produce IL-12 or IL-10 depending upon whether the animals are undergoing self-healing (Th1-associated) or progressive (Th2-associated) disease, respectively (12, 35). The depletion of granulocytes in Candida-infected mice undergoing infection with a normally self-healing yeast strain leads to the development of a Th2 response and progressive disease. Thus, by virtue of their ability to produce two key immunomodulatory cytokines, neutrophils exert a major impact in determining whether or not the host survives C. albicans infection.

We do not yet know if PMN play such a critical immunoregulatory role during early T. gondii infection. The ability of the host to survive T. gondii infection is critically dependent upon IL-12 and subsequent Th1 development (16, 27, 36–39). Studies in mice indicate that neutrophil depletion increases the susceptibility to acute toxoplasmosis (22, 23), and while the reasons for this are unclear, it is plausible that early neutrophil IL-12 production contributes to the protective activity of these cells. Nevertheless, monocytes/macrophages and dendritic cells are also capable of T. gondii-induced IL-12 synthesis, and it is probable that all three cell types are active in IL-12 production during the first contact of Toxoplasma with the innate immune system.

The stimulation of human neutrophils with T. gondii also resulted in the rapid induction of MIP-1 mRNA, and the up-regulation of transcripts was sustained for at least 12 h. The ability of neutrophils to produce MIP-1 proteins has been previously reported (13, 14, 33), although chemokine expression induced by Toxoplasma itself is relatively little studied. In this regard, the mouse CXC chemokines MuMig and Crg-2 are induced in dependence upon IFN-γ during T. gondii infection, although the functional consequences resulting from the expression of these T cell chemoattractants are not known (40).

The MIP-1 cytokines themselves are related CC-family chemokines that display extensively overlapping biological activity (41). Both MIP-1α and MIP-1β display chemotactic activity for macrophages, NK cells, and dendritic cells (28, 30, 42, 43). The synthesis of MIP-1α and MIP-1β is associated with type 1 immune responses (44). Indeed, it was recently reported that MIP-1α and MIP-1β serve as chemoattractants for Th1, but not Th2, cells (45, 46). Therefore, the strong type 1 cytokine response induced by T. gondii would predict the up-regulation of parasite-induced MIP-1 gene transcription, which we report here. We are presently employing mouse model systems to more closely examine the role of these chemokines during infection.

The role of IFN-γ in neutrophil chemokine responses is complex (32, 47). Thus, the cytokine appears to display an inhibitory activity on neutrophil MIP-1 expression, as measured by gene transcript induction and protein expression. However, during extended incubation, IFN-γ has the opposite effect, promoting neutrophil MIP-1 expression. IFN-γ also appears to be a potent inhibitor of
MIP-1α and MIP-1β expression by thioglycollate-elicited macrophages stimulated with hylauronan (48). In our experiments, the addition of IFN-γ did not inhibit chemokine transcript levels, and, indeed, their levels appeared slightly augmented. Because STAg and LPS displayed distinct TNF-α induction patterns (e.g., Fig. 2), it may also be that these chemokines are regulated differently when triggered by alternate stimuli. We are currently further examining the role of IFN-γ on neutrophil chemokine expression.

The cytokine TNF-α has previously been shown to induce the expression of several neutrophil cytokines, including MIP-1α and IL-8 (15, 49). We also found that TNF-α alone displayed potent MIP-1α- and MIP-1β-inducing activity in PMN. Our finding that STAg also induces PMN TNF-α production raises the possibility that MIP-1 expression in these cultures is an indirect activity of STAg resulting from autocrine stimulation by TNF-α. Indeed, the addition of a neutralizing anti-TNF-α antiserum blocked increases in MIP-1α and MIP-1β gene transcript levels. Nevertheless, up-regulation of the MIP-1 genes was never completely eliminated and it is, therefore, possible that there remains a TNF-α-independent component to STAg-induced MIP-1 gene induction. We are currently further examining this issue.

Neutrophils are present in high numbers in the peripheral blood and in response to infection rapidly exit the circulation and accumulate at sites of tissue damage or infection. The finding that PMN are capable of producing several major inflammatory cytokines in response to T. gondii suggests that neutrophils may be important in directing early cell trafficking and cytokine-producing activities during infection with this microbial pathogen. Thus, a scenario that we favor is that PMN initially migrate to a focus of infection, where they are stimulated to produce chemokines that subsequently serve an instrumental role in recruiting cells such as monocytes and dendritic cells during early infection. The activation of these cells, in turn, could be accomplished by the combined stimuli of parasites and neutrophil-derived cytokines.

References


