Release of Interleukin-1 and Interleukin-6 From Human Monocytes by Antithymocyte Globulin: Requirement for De Novo Synthesis

By Panchala Rameshwar and Pedro Gasçon

Antithymocyte globulin (ATG) is an effective treatment in patients with severe aplastic anemia (SAA). Its mechanism of action remains unclear, although it has been assumed to be immunosuppressive. However, ATG also has been shown by several laboratories to be immunostimulatory. Recently, interleukin-1 (IL-1) production has been found to be decreased in lipopolysaccharide-stimulated peripheral blood monocytes obtained from SAA patients. We have investigated the ability of ATG to function as an immunostimulatory agent via the production of IL-1 and IL-6 by normal human monocytes in vitro. Supernatants from ATG-stimulated monocytes were assayed for biologically active and immunoreactive IL-1 and IL-6. We have found that ATG, via its F(ab')2 fragment is a powerful inducer of IL-1 and IL-6 production. Furthermore, ATG induction of both cytokines from normal monocytes required de novo synthesis, as determined by 35S-methionine incorporation. Because these two cytokines synergize with other cytokines at both the stem cell and progenitor levels, these stimulatory properties of ATG may be relevant to the treatment of SAA. This would favor the hypothesis of a bimodal mechanism for ATG as an inducer of hematopoietic growth factors and as an immunosuppressive agent.

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Antilymphocyte globulins (ALG) and antithymocyte globulins (ATG) are equine immune sera prepared against pooled human lymphocytes and thymocytes, respectively. Both are active agents in the treatment of severe aplastic anemia (SAA) and their effectiveness has been confirmed by several clinical trials in which 40% to 75% of patients with SAA became transfusion-independent or had significant hematologic responses leading to increased survival.1-8 The mechanism of action of both preparations remains unknown, although it is assumed to be immunosuppressive. However, in vitro, both ATG and ALG have been shown to have immunostimulatory properties.9-13 They can stimulate T cells to proliferate9,12,16 and to induce the production of hematopoietic growth factors from peripheral blood mononuclear cells (PBMCs): granulocyte-macrophage colony-stimulating factor (GM-CSF)12-14 and interleukin-3 (IL-3).12,14 ATG and ALG have also been shown to stimulate bone marrow MNCs (BMMNCs) to proliferate in tissue culture.10,15,17 Several laboratories have found an impairment in the production of IL-1 by monocytes from patients with SAA.18-20 It is possible that in some cases of SAA, an immunosuppressive mechanism and/or an autoimmune process may coexist with an insufficient stimulation of progenitor cells secondary to decreased production of hematopoietic growth factors (HGFs). ATG stimulates immune cells such as lymphocytes and monocytes to release cytokines with immunologic regulatory properties on the hematopoietic system.18,21 Therefore, it is possible that in some cases of SAA, the mechanism of ATG may be dual, ie, immunosuppressive and immunostimulatory, both necessary to restore hematopoiesis.

We have studied the capability of ATG to induce the production from normal human monocytes of two cytokines that are known to act at both the stem cell and progenitor levels, IL-1 (decreased in SAA patients) and IL-6.

MATERIALS AND METHODS

Cell lines. D10.G4.1 and CTLL-2, IL-1- and IL-2-dependent cell lines, respectively, were purchased from the American Type Culture Collection (ATCC, Rockville, MD). The B9 hybridoma,22 a human IL-6-dependent cell line, was kindly provided by Dr R.F. Nordan (National Institutes of Health, Bethesda, MD).

Cytokines and antibodies. Human recombinant IL-1α (rhIL-1α) and anti–hIL-1α, and anti–hIL-1β, were purchased from Endogen (Boston, MA). Monoclonal murine anti–hIL-1α and anti–hIL-1β were purchased from Collaborative Biomedical (Bedford, MA). rhIL-6 and murine anti–hIL-6 were generously provided by Genetics Institute (Cambridge, MA). Goat polyclonal anti–hIL-6 and murine monoclonal anti–hIL-6 were purchased from R&D (Minneapolis, MN) and Collaborative Biomedical, respectively. Biotinylated antimouse IgG was purchased from Vector Laboratories (Burlingame, CA). Anti-Mo2 (CD14) was obtained from ascites derived from the hybridoma, HB-44 (ATCC), grown in mice. Affinity-purified ascites was prepared by binding to a protein G-Sepharose (Sigma, St Louis, MO) column. The optimum working concentration of the purified antibody was determined by fluorescence-activated cell sorter (FACS) analysis with purified human monocytes (1/200 final dilution of the antibody at 25 mg/mL).

Cell separation. PB was collected from normal subjects after informed consent. Adherent cells were separated using a modification of the method described by Freundlich and Avidalovic.23 Briefly, PBMCs and plasma were separated by Ficoll® (Pharmacia, Piscataway, NJ) density gradient centrifugation. Autologous platelet-free plasma was prepared by high-speed centrifugation (1,000g for 15 minutes) and then incubated on endotoxin-free, tissue culture grade gelatin-coated (Sigma, St Louis, MO) column. The optimum working concentration of the purified antibody was determined by fluorescence-activated cell sorter (FACS) analysis with purified human monocytes (1/200 final dilution of the antibody at 25 mg/mL).

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flushing the dishes with cold deadherent medium. Greater than 95% of the adherent cells (monocytes) were positive for nonspecific esterase and expressed the CD14 antigen, as determined by FACS analysis.

**Stimulation of monocytes.** Normal human monocytes were plated in 24-well tissue culture dishes (Falcon 3047) at 1 × 10^6 cells/mL in serum-free RPMI 1640 medium. Cells were stimulated for 8 hours with nonimmune horse IgG (10 μg/mL), nonimmune IgG; US Biochemical Co, Cleveland, OH), ATG (10 μg/mL gift of Upjohn, Kalamazoo, MI), lipo polysaccharide (LPS), Salmonella typhosa (2 μg/mL Sigma), polymyxin B and nonimmune IgG, polymyxin B and ATG, or polymyxin B and LPS. Similar concentrations of monocytes incubated with medium alone were used as controls. After incubation, supernatants (SNs) were cleared via centrifugation (4,000g for 5 minutes), aliquoted, and stored at −22°C until ready to be assayed.

**Cytokine level estimation.** IL-1 and IL-6 activities were determined in biologic assays and by the enzyme-linked immunosorbent assay (ELISA).

IL-1 activity in the SNs was determined based on the ability to support the growth of concanavalin A (Con A; Sigma)-stimulated mouse helper T-cell clone, D10.G4.1. Maintenance of the D10.G4.1 cell line has been previously described. Briefly, cells were cultured in the presence of Con A-stimulated rat spleen cells SN and then expanded via activation by egg white conalbumin (US Biochemical) in the context of γ-irradiated I-A^B (AKR/J spleen) feeder cells. Assays were performed at least 8 days after stimulation (quiescent cells). SNs (0.1 mL) in triplicate were serially diluted in Click's medium (Irvine Scientific, Santa Ana, CA) containing 5% FCS and 2.5 μg/mL Con A (assay medium) in microtiter wells (Falcon 3072). For each assay, a standard curve was established using serial dilutions of rhIL-1α or rhIL-1β at 500 U/mL. Quiescent cells (2 × 10^6), resuspended in 0.1 mL assay medium, were added to each well. After 68 hours (according to ATCC recommendations), wells were pulsed for 4 hours with 1 μCi tritiated thymidine ([³H]Tdr, 2 Ci/μmol; New England Nuclear, Boston, MA). Cells were harvested onto glass fiber filters with a PHD cell harvester (Cambridge Technology, Cambridge, MA) and [³H]Tdr incorporation was determined by liquid scintillation counting. SNs were tested for the presence of IL-2 in a proliferative assay with the CTLL-2 cell line as described. IL-6 activities were determined based on the proliferative response of the B9.9 cells. SNs (0.1 mL), in triplicate, were serially diluted in RPMI 1640 containing 10% FCS in microtiter wells and then incubated with 2 × 10^6 cells per well. A standard curve was established with serial dilutions of rhIL-6 at 500 U/mL. After 72 hours, wells were pulsed with 1 μCi [³H]Tdr and further incubated for an additional 4 hours. Cells were harvested onto glass fiber filters with a PHD cell harvester. For all biosassays, 1 cytokine unit was equivalent to one half-maximal growth of the cells as determined by [³H]Tdr incorporation in the standard curves (standard cytokines were kindly provided by Dr Craig W. Reynolds, National Cancer Institute, National Institutes of Health, Bethesda, MD).

The presence of biologically active cytokines was quantitated by a modification of the sandwich ELISA as previously described. Capture and second antibodies were added at 4 μg/mL in Tris buffer (pH 8.9), and PBS containing 3% bovine serum albumin (BSA), respectively. Binding of the second antibody was detected by incubating with a specific biotinylated tagged antibody amplified with Avidin D (20 μg/mL Vector), followed by incubation with biotinylated conjugated alkaline phosphatase (0.2 U/mL Vector). Bound enzyme was detected with Sigma 104 phosphatase substrate, which was dissolved in 0.5 mmol/L diethanolamine buffer containing 0.5 mmol/L magnesium chloride, and 0.02% sodium azide (all from Sigma). Color development was measured at 405 nm on a Mdl 450 microplate reader (BioRad, Richmond, CA).

**Western blots.** Protease inhibitors phenylmethylsulfonyl fluoride (PMSF; 1 μmol/L), leupeptin (5 μmol/L), chymostatin (1 μg/mL), and (3-[3-Cholamidopropyl]-dimethylammonio)-2-hydroxy-1-propanesulfonate) (0.1 μmol/L) were added to SNs, which were then centrifuged 10 × with a Centrifuge 10 microcentrifuge (Amicon, Danvers, MA). Immunoblotting was performed at room temperature using a modification of the method described by Towbin et al. Briefly, proteins were separated on 16% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then electrochemically transferred to Immobilon PVDF transfer membrane (Millipore, Bedford, MA) for 45 minutes at a constant current of 400 mA and 50 V. Protein-bound membranes were incubated with PBS containing 2% BSA (US Biochemical) for 2 hours and then incubated overnight with the following primary antibodies: 100 neutralizing units (NU) rabbit anti-hIL-1α or anti-hIL-1β or 10 μg goat anti-hIL-6. Sixteen to 24 hours later, excess antibodies were washed three times (15 minutes each) with 2% BSA in PBS. IL-1α and IL-6 activities were incubated for 1 hour with horseradish peroxidase-conjugated goat antirabbit IgG (BioRad) and alkaline phosphatase-conjugated swine antigoat IgG (Boehringer-Mannheim, Indianapolis, IN), respectively. Color was developed with the appropriate substrate. For all gels, the molecular weights (MWs) of the developed bands were compared with pre-stained low MW standards (Diversified Biotech, Newton Centre, MA).

**De novo synthesis.** Monocytes (1 × 10⁶/mL) were resuspended in methionine-free RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 1 μCi/Ci/mL [³⁵S]methionine (1,000 Ci/mmol, ICN, Irvine, CA) and 1× serum supplement (GMS-A; GIBCO). Cells were stimulated for 16 hours with nonimmune IgG or ATG, or cultured in medium alone. SNs were cleared by centrifugation (4,000g for 5 minutes) and then the following protease inhibitors were immediately added: PMSF (1 μmol/L), leupeptin (5 μmol/L), chymostatin (1 μg/mL), and (3-[3-Cholamidopropyl]-dimethylammonio)-2-hydroxy-1-propanesulfonate) (0.1 μmol/L). SNs were incubated for 5 hours at 4°C with rabbit anti-hIL-1α or anti-hIL-1β. Sixteen to 24 hours later, excess antibodies were washed three times (15 minutes each) with 2% BSA in PBS. IL-1α and IL-6 activities were incubated for 1 hour with horseradish peroxidase-conjugated goat antirabbit IgG (BioRad) and alkaline phosphatase-conjugated swine antigoat IgG (Boehringer-Mannheim, Indianapolis, IN), respectively. Color was developed with the appropriate substrate. For all gels, the molecular weights (MWs) of the developed bands were compared with prestained low MW standards.

**Preparation of F(ab′)+2 andFc fragments.** Both nonimmune IgG and ATG were split into their F(ab′)+2 and Fc fragments by pepsin (Worthington Biochemical, Freehold, NJ) digestion. We used a modification of the method previously described by Kurtela et al. 23 F(ab′)+2 fragments were prepared by overnight (undetermined) digestion of IgG. After this period, most of the Fc portions of the molecules were degraded. The remaining Fc and intact IgG molecules were separated from the F(ab′)+2 fragments by binding to a protein G-Sepharose (Sigma) column. The F(ab′)+2 fragments were collected in the eluent and concentrated with a 30-KD MW.
cut-off Amicon ultrafiltration cell. The final protein concentration of the F(ab')2 solution (0.18 mg/mL) was determined by the method described by Bradford using BSA as standard (the standard and dye solution were both obtained from BioRad). Purity of the F(ab')2 fragments was confirmed by SDS-PAGE under both reducing and nonreducing conditions. Fc fragments were prepared by digesting with pepsin for 2 hours. After this period, the percentage of intact molecules was far greater than the amount of Fc molecules. The F(ab')2 fragments were separated from the Fc and IgG molecules by binding the two latter molecules to a protein G-Sepharose column. Both Fc and intact IgG molecules were eluted with 0.1 mol/L glycine buffer (pH 2.7). The Fc fragments were then separated from the IgG molecules by size-exclusion gel chromatography on a Sephacryl G25 (Pharmacia) column. Fc molecules were not degraded as determined by gel electrophoresis on 12.5% SDS-PAGE under nonreducing conditions. The fraction that contained the Fc fragments was concentrated with a 10-Kd MW cut-off Amicon ultrafiltration cell. The final protein concentration of the Fc solution (0.1 mg/mL) was determined by the method described by Bradford.

Statistical analysis. Data were analyzed using the Student's t-test to determine the significance (P value) or differences between experimental and control SNs.

RESULTS

Cytokine release by human PB monocytes. In eight separate experiments, two different lots of ATG (844JX and 313BH) were used to stimulate normal human monocytes. The amounts of biologically active IL-1 and IL-6 released were 27-fold and 100-fold, respectively, above the control cut-off. The t-test was used to determine the significance (Table 1A). The amounts of each cytokine from ATG-stimulated monocytes were 10- to 15-fold above control (monocytes cultured with medium alone), whereas the levels induced by nonimmune horse IgG were comparable to control levels.

ATG directly stimulates the release of IL-1 and IL-6. Because IL-1 had been reported to stimulate the release of IL-6, we investigated whether IL-6 was being released directly by ATG or indirectly via IL-1. Monocytes were cultured in the presence of equivalent concentrations of ATG, nonimmune IgG, or ATG and NU anti-hIL-1a and anti-hIL-1b.

Table 1. IL-1 and IL-6 Production by ATG-Stimulated Human Monocytes

<table>
<thead>
<tr>
<th></th>
<th>ELISA (ng/mL, mean ± SD)</th>
<th>Biologic Assay (units, mean ± SE)</th>
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<tbody>
<tr>
<td></td>
<td>IL-1a</td>
<td>IL-1b</td>
</tr>
<tr>
<td>Medium (n = 3)</td>
<td>8 ± 3</td>
<td>13 ± 3</td>
</tr>
<tr>
<td>Horse IgG (nonimmune) (n = 3)</td>
<td>15 ± 5</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>ATG (n = 6)</td>
<td>122 ± 14</td>
<td>205 ± 20</td>
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<tr>
<td></td>
<td>IL-6</td>
<td></td>
</tr>
<tr>
<td>Medium (n = 8)</td>
<td>15 ± 4</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>Horse IgG (nonimmune) (n = 8)</td>
<td>18 ± 2</td>
<td>13 ± 2</td>
</tr>
<tr>
<td>ATG (n = 8)</td>
<td>400 ± 30*</td>
<td>1,380 ± 170*</td>
</tr>
<tr>
<td>ATG + NU IL-1a + b (n = 5)</td>
<td>10 ± 2</td>
<td>1,436 ± 19</td>
</tr>
<tr>
<td>ATG + NU IL-6 (n = 5)</td>
<td>426 ± 23</td>
<td>59 ± 4</td>
</tr>
<tr>
<td>NU IL-1a + b (n = 5)</td>
<td>6 ± 3</td>
<td>20 ± 2</td>
</tr>
<tr>
<td>NU IL-6 (n = 5)</td>
<td>8 ± 1</td>
<td>17 ± 1</td>
</tr>
</tbody>
</table>

Release of IL-1 and IL-6 from human PB monocytes. Monocytes were cultured in serum-free medium alone or stimulated with nonimmune horse IgG, ATG, ATG and anti-hIL-1a and b, ATG and anti-hIL-6, anti-hIL-1a and b, and ATG and anti-hIL-1a and IL-6. SNs were tested for IL-1a and IL-6 as described in Materials and Methods. (A) cytokine concentration (ng/mL); (B) biologic activity (units/mL).

*P < .05.
ATG-induced production of IL-1 and IL-6 by human monocytes is not due to endotoxin contamination. Monocytes were incubated in serum-free medium alone, or stimulated with LPS, LPS and polymyxin B, or ATG in the presence or absence of polymyxin B. Supernatants were tested for both IL-1 and IL-6 as described in Materials and Methods.

from nonimmune IgG or ATG. Fc cocultured with monocytes did not produce either IL-1 or IL-6 above control levels, whereas the F(ab')2 induced the release of a similar quantity of both interleukins as compared with cells stimulated with the same concentration of the intact ATG (Fig 2).

Confirmation of biologically active cytokines. Because most cell lines used in biologic assays have been shown to respond to various cytokines, the presence of IL-1α, IL-1β, or IL-6 in the SNs of ATG-stimulated monocytes was confirmed immunologically via Western blots with specific antibodies. Lanes with ATG-stimulated SNs resulted in a band at 17 Kd for membranes blotted with anti-hIL-1α and anti-hIL-1β (Fig 3A and B, respectively) and 22 Kd for anti-hIL-6-blotted membranes (Fig 3C). Lanes with cells cultured with medium or stimulated with nonimmune IgG showed no detectable bands in the case of immunoblots of IL-1α and IL-1β, and a low level of immunoprecipitates was observed for IL-6. Controls include lanes with purified IL-1α, IL-1β, and IL-6 (Fig 3A, B, and C, respectively).

De novo synthesis. Transcription of the IL-6 gene has been shown to be initiated by adherence to plastic. Because the technique used to separate monocytes involves prolonged adherence to plastic coated with gelatin incubated with autologous plasma, we investigated whether there was a requirement for de novo synthesis for IL-1α, IL-1β, or IL-6 in the SNs obtained from cells stimulated with either ATG or nonimmune IgG or cells cultured with medium alone. In all experiments, culture medium included 35S-methionine-supplemented methionine-free medium. SNs obtained from cultures stimulated with ATG and immunoprecipitated with either anti-hIL-1α or anti-hIL-1β resulted in a band at 17 Kd after autoradiography (Fig 4A), whereas a band at 22 Kd was seen with anti-hIL-

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**Fig 1.** ATG-induced production of IL-1 and IL-6 by human monocytes is not due to endotoxin contamination. Monocytes were incubated in serum-free medium alone, or stimulated with LPS, LPS and polymyxin B, or ATG in the presence or absence of polymyxin B. Supernatants were tested for both IL-1 and IL-6 as described in Materials and Methods.

**Fig 2.** Effect of the F(ab')2 and Fc fragments of the ATG molecule on human monocytes. Monocytes were stimulated with the intact nonimmune IgG, ATG, or a similar concentration of F(ab')2 or Fc fragments of both nonimmune IgG or ATG in serum-free medium. Supernatants were tested for both IL-1 and IL-6 as described in Materials and Methods.
INTERLEUKIN RELEASE BY ANTITHYMOCYTE GLOBULIN

Fig 3. Western blot analysis of supernatants from monocytes stimulated with ATG or nonimmune IgG. Supernatants were separated on 16% SDS-PAGE (see Materials and Methods). Lane 1, ATG; lane 2, nonimmune IgG; lane 3, medium; lane 4, standard cytokines: 20 ng rhIL-1α (A), 5 ng rhIL-1β (B), 10 ng rhIL-6 (C); lane 5, 5 μL prestained low MW standard.

Neither IL-1 nor IL-6 was detected with SNs obtained from monocytes cultured with medium or stimulated with nonimmune IgG (Fig 4A and B).

DISCUSSION

The mechanism of action of ATG is thought to be immunosuppressive. This is in part due to its in vivo cytotoxic effect on lymphocytes during ATG therapy, particularly the elimination of an activated lymphocyte population.\(^6\) Hematologic responses seen with high-dose methylprednisolone\(^6\) and more recently with cyclosporine A,\(^4\) two known immunosuppressive agents, suggest that ATG may function primarily, in SAA patients, as an immunosuppressive agent. However, in vitro studies from different laboratories have also placed ATG as an immunostimulatory agent.\(^7\) Our results show that ATG induces the release of both IL-1 and IL-6 by highly purified normal human monocytes (Table 1). This response was not due to the presence of endotoxin contamination because the presence of polymyxin B in coculture with ATG did not alter the values of IL-1 and IL-6 (Fig 1). The inability of polymyxin B to alter the results of ATG cannot be attributed to insufficient quantity because the same concentration of polymyxin B was able to block the effects of the optimum concentration of LPS (Fig 1).

The stimulatory action of ATG on human monocytes was mediated by the F(ab')\(_2\) segment of the molecule and not by the Fc component (Fig 2). This is further supported by the inability of syngeneic nonimmune IgG to stimulate monocytes to release IL-1 and IL-6 (Table 1). In addition, these biologic effects observed were not due to thimerosal, the preservative present in the ATG preparations,\(^3\) because affinity-purified ATG (protein G sepharose) shows the same activity as the intact preparation (results not shown).

Neutralizing units of IL-1 in coculture with ATG did not alter the values of IL-6, implying that the release of IL-6 by ATG was not due to autostimulation by the released IL-1. Also, the release of both IL-1 and IL-6 was not due to nonspecific stimulation of monocytes by opsonization because equivalent Ig concentrations of anti–IL-6, anti–IL-1α, and anti–IL-1β incubated with monocytes did not stimulate the release of IL-6 or IL-1 (Table 1). The presence of NU anti–IL-6 in the ATG-stimulated cultures did not reduce the B9.9 proliferation to background unless anti–GM-CSF was added. This may be due to the ability of the B9.9 cells to respond to GM-CSF present in the cultures, even though others have shown that the B9.9 cells do not respond to GM-CSF,\(^4\) a cytokine released by ATG-stimulated monocytes (manuscript in preparation). It is also possible that GM-CSF present in the SNs may be inducing the release of another growth factor capable of inducing the B9.9 cells to proliferate.

Finally, our results indicate that ATG-induced production of IL-1 and most of the IL-6 requires de novo synthesis. In the latter case, faint bands are detected in the control lanes of the Western blots (Fig 3). The presence of these faint bands may be explained by amplification of back-
ground cytokine levels due to concentration of SNs (×12). Therefore, it seems that the majority of the induced cytokines are due to ATG stimulation and not a consequence of monocyte adhesion to plasma-gelatin–coated plastic. However, the possibility of nonspecific transcription of both the IL-1 and IL-6 genes cannot be excluded because neither the presence of the respective messenger RNA nor the requirement for ATG-mediated transcription were studied. Therefore, at this time, we cannot conclude whether ATG is regulating the release of IL-1 and IL-6 at the transcriptional or translational levels.

Several laboratories have recently reported a decreased production of IL-1 by monocytes from patients with SAA. In a recent study, stromal macrophages from mice exposed to benzene (BZ), a hematotoxin known to cause aplastic anaemia, were unable to process pre–IL-1 to the mature biologically active cytokine. Furthermore, administration of rIL-1 to mice before BZ completely prevented BZ-induced myelotoxicity. These results strongly suggest that decreased HGFs such as IL-1 may be either a cause or a coexistent cause for some cases of SAA. ATG may induce the production of IL-1 in these patients by either stimulating SAA monocytes directly or via other IL-1–producing cells, because it is known that ATG binds to multiple types of cells. ATG, by stimulating the defective monocytes of SAA, may partially restore a possible growth factor imbal-

Fig 4. (A) Requirement for de novo synthesis of IL-1α and IL-1β determined by 35S-methionine uptake. Monocytes were cultured with ATG or nonimmune IgG. Immunoprecipitated (IL-1α and IL-1β), concentrated cytokines were separated on 16% SDS-PAGE, dried, and then developed by autoradiography (see Materials and Methods). Lane 1, ATG/IL-1α; lane 2, ATG/IL-1β; lane 3, nonimmune horse IgG; lane 4, medium. (B) Requirement for de novo synthesis of IL-6 determined by 35S-methionine uptake. Monocytes were cultured as per (A). Supernatants were incubated with goat anti-hIL-6. Immune complexed IL-6 were immunoprecipitated by incubating with protein A sepharose (see Materials and Methods). Lane 1, ATG; lane 2, nonimmune IgG; lane 3, medium.
ance either directly by inducing the release of HGFs by the same monocytes or indirectly by the action of IL-1 and IL-6 on BM accessory cells. A possible indirect mechanism may be the initiation of a cascade effect by the released IL-1 and IL-6 in which other HGFs can be released to further stimulate residual hematopoiesis. In fact, recent studies have shown that both ATG and ALG can induce the release of GM-CSF and IL-3 by PBMCs and T cells. Growth factors that may be partially released by IL-1, \(^{1,2,4}\) Recent clinical trials with HGFs (rhG-CSF, \(^{45,46}\) rhGM-CSF, \(^{47}\) and IL-3\(^ {31}\)) in patients with SAA have been shown to induce transient hematopoietic responses. \(^{49-51}\) The return to baseline after discontinuation of the growth factor treatment suggests that hematopoietic suppression can be overridden by stimulatory factors. It is also possible that the transient BM recovery observed with this type of treatment may be due to the replacement of a particular HGF deficient in some SAA patients. The observed production of IL-1 and IL-6 by ATG-stimulated human monocytes may be relevant to the treatment of SAA and further supports the role for ATG in stimulating hematopoiesis.

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