Impaired Mononuclear Cell Tissue Factor Generation in Patients With Immunodeficiency Diseases

By Mark Ballow and Frederick R. Rickles

Congenital immunodeficiency diseases are complex disorders of B- and T-cell function. We have studied monocyte-lymphocyte interaction in four patients with severe immunodeficiency disease using the coagulation protein, tissue factor, as a marker for monocyte activation. Phytohemagglutinin-induced tissue factor activity was markedly reduced in the cells from all four patients as compared to controls (p < 0.02). Lipopolysaccharide-induced tissue factor activity, however, was abnormal in two patients with defects in stem cell differentiation (p < 0.00001), but was normal in two patients with thymic hypofunction. Immune reconstitution of one patient with adenosine deaminase deficiency by bone marrow transplantation was marked by the return of mononuclear cell tissue factor activity to normal. Coculture experiments in three patients revealed suppression of tissue factor activity in two of the patients. Inhibitory activity gradually disappeared in the transplanted child as normal immune function was reconstituted. We conclude that mononuclear cell tissue factor activation is, in part, under immune control, in that T-cell function is required for optimal generation of activity, and immune reconstitution corrects the deficiency. Moreover, we have supplied further evidence for the interaction of blood coagulation with cell-mediated immunity by this demonstration of defective mononuclear cell tissue factor generation in children with immunodeficiency disease.

SEVERE COMBINED immunodeficiency diseases (SCID) are comprised of a heterogeneous group of patients exhibiting a spectrum of deficiencies of both T- and B-cell immune function. Appreciation of the complexity of these disorders is reflected by the variability of the genetic inheritance, clinical manifestations, and the immunologic findings. More recently, studies of the differentiation of lymphoid precursor cells in the bone marrow in the presence of adenosine deaminase (ADA) enzymes, thymic humoral factors, or thymic epithelial monolayers have suggested that the heterogeneity of these disorders is related to various types of blocks or defects of stem cell differentiation.

However, the study of SCID patients has mainly focused on the lymphoid system and immune function. The monocyte-macrophage system has received less attention in SCID, although of major importance in the normal host defense mechanisms. In addition to the basic functions of microbial phagocytosis and killing, the monocyte is involved in the afferent limb of the immune response of antigen processing and presentation, and in the efferent limb by virtue of regulation of T- and B-cell immune responses, both by direct cell–cell interaction and the secretion of soluble mediators.

A newly recognized function of the monocyte-macrophage system is the elaboration of the blood coagulation protein tissue factor from mononuclear cells. Mononuclear cell tissue factor (MCTF) or thromboplastin, which is the same lipophilic and membrane-associated glycoprotein found in abundance in human lung, brain, and placenta, is important in the initiation of the extrinsic coagulation system. A variety of stimuli of immunologic interactions, including endotoxin (lipopolysaccharide, LPS), mitogens and specific antigens, allogeneic cells in mixed lymphocyte culture (MLC), and immune complexes, can induce the activation of MCTF, which suggests that the coagulation system may play a role in lymphocyte-mediated processes. Recently, Edwards, Rickles, and Bobrove examined MCTF activation in human mononuclear cells fractionated into several subpopulations. These studies demonstrated that: (1) monocyte-phagocytes are the cells responsible for MCTF generation; (2) LPS can stimulate monocytes to generate MCTF in the absence of T and/or B cells, but optimal generation does require the presence of T cells; (3) MCTF generation in response to phytohemagglutinin (PHA) requires the presence of T cells (or a lymphokine). Rickles and Bobrove (submitted for publication) have suggested that B cells do not play a significant role in MCTF generation, since normal production of MCTF was found in a patient with agammaglobulinemia and absence of B cells. The potential role of blood coagulation proteins, and tissue factor in particular, in the mediation of delayed hypersensitivity reactions has been reviewed recently.

We present four patients with severe immunodeficiency diseases, two of whom had SCID. Our studies suggested that MCTF generation is more related to the thymus or thymic processes than to peripheral lymphoid structures and that MCTF is a convenient marker for evaluating immunologic function.
ciency disease who have impaired MCTF generation. Using endotoxin and PHA as activators of MCTF, a spectrum of defects in MCTF generation was demonstrated. The most severe forms of SCID demonstrated defective MCTF generation in response to both LPS and PHA. Patients with predominant T-cell abnormalities demonstrated a marked defect with PHA but only minimal abnormalities with LPS. Mononuclear cells from two of the SCID patients, including one with ADA deficiency, demonstrated inhibition of MCTF activity when their cells were cocultured with normal mononuclear cells. In the patient with ADA deficiency, immune reconstitution following bone marrow transplantation was associated with the return of MCTF activity to normal levels in response to both LPS and PHA. These observations suggest that the pathogenesis of SCID affects a specific monocyte function as well as T- and B-cell immune function.

MATERIALS AND METHODS

Patients

Four individuals with immunodeficiency diseases were studied. Patient 1 had severe combined immunodeficiency disease (SCID) with adenosine deaminase deficiency. The patient was studied on several occasions before and after successful bone marrow transplantation from an HLA- and MLC-matched sibling. Patient 2 has SCID with demonstrable circulating B cells and an in vitro response to allogeneic cells in the MLC. Patient 3 has thymic hypoplasia, and complete details of the clinical course are reported elsewhere. The essential immunologic studies of these 4 patients are presented in Table 1.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age/sex</td>
<td>8 mo/female</td>
<td>9-15 mo/male</td>
<td>20 mo/female</td>
<td>15 yr/male</td>
</tr>
<tr>
<td>Immunoglobulin concentration (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>310</td>
<td>150</td>
<td>270</td>
<td>792</td>
</tr>
<tr>
<td>IgA</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>18</td>
<td>63</td>
</tr>
<tr>
<td>IgM</td>
<td>7</td>
<td>31</td>
<td>234</td>
<td>216</td>
</tr>
<tr>
<td>Specific antibody production to</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isohemagglutinins</td>
<td>0</td>
<td>0</td>
<td>1:8</td>
<td>1:8</td>
</tr>
<tr>
<td>Viruses</td>
<td>0</td>
<td>Polio</td>
<td>Polio (+)</td>
<td>Rubella (+)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type I-1:8</td>
<td>Type II-1:128</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rubella (+)</td>
<td></td>
<td>Influenza (+)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Varicella (+)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td>0</td>
<td>Tetanus (+)</td>
<td>Tetanus (+)</td>
<td>Tetanus (+)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Meningococcus</td>
<td>Diptheria (+)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A and C (+)</td>
<td></td>
<td>Pneumococcus (+)</td>
</tr>
<tr>
<td>Absolute lymphocyte count (cells/cu mm)</td>
<td>400</td>
<td>1,200</td>
<td>700</td>
<td>1,168</td>
</tr>
<tr>
<td>Delayed skin hypersensitivity</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Candida (+)*</td>
</tr>
<tr>
<td>(including sensitization to DNCB)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell markers</td>
<td>E-rosette-forming cells</td>
<td>1%-8%</td>
<td>8%-14%</td>
<td>28%</td>
</tr>
<tr>
<td>(Normal controls = 64.6% ± 2.6%)‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EAC-rosette-forming cells</td>
<td>80%</td>
<td>18%</td>
<td>25%</td>
<td>13%</td>
</tr>
<tr>
<td>(Normal controls = 28.4% ± 5.6%)‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blastogenic response to</td>
<td>Mitogens‡ (% of normal)</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Con-A ~ 33%*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specific antigens</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Allogeneic cells in MLC§</td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
<td>Present</td>
</tr>
</tbody>
</table>

*When initially studied, all of these tests were abnormal. Transfer factor therapy was initiated prior to the performance of the tests and resulted in partial return of T-cell function.
†Mean ± 1 SD.
‡Mitogens, PHA and Con-A.
§MLC, mixed lymphocyte culture reaction.
µg/ml, respectively, to stimulate the cells. Cell cultures were incubated in a humidified atmosphere of 5% CO2, 95% air and were harvested by sonication (4°C for 20 sec; Bioso Inc., Rochester, NY) after 18 hr of incubation. The tissue factor content of whole culture sonicates was assayed by the one-stage method described in detail elsewhere. Briefly, cell sonicates were evaluated for tissue factor procoagulant activity by a modification of the one-stage activated partial thromboplastin time test (APTT). Fresh-frozen normal pooled human plasma depleted of factors XII and XI was used as substrate, and automated APTT reagent (General Diagnostics Laboratories, Morris Plains, N.J.) was used as a source of mixed brain lipids. Since previous studies had established that in these cultures only the subpopulation of adherent cells that ingests latex particles generate MCTF, activity is expressed per monocyte as compared to an arbitrary standard tissue factor preparation (rabbit brain thromboplastin, Dade Products, Miami, Fla.) Specificity of this assay for tissue factor has been established.

Coculture experiments were handled in a similar manner. Cells from the patients were mixed in an equal proportion with cells from a panel of normal donors and stimulated as described. Controls for inhibitory activity in these experiments consisted of (1) similar mixtures of 2 different normal donors, and (2) cells from normal donors diluted to 50% concentration (5 x 10^6 cells/ml) with RPMI 1640. Each of these control cultures was stimulated with LPS. A significant decrease in tissue factor activity could not be demonstrated in either of these controls, and therefore, inhibitory activity produced by the patient cells was expressed as percent inhibition of the MCTF activity generated by normal donor cells in response to the same stimulus (LPS). "Controls" consisted of 16 experiments performed in 9 normal laboratory volunteers concurrent with the patient studies. Although age-matched controls were not often available, a limited number of separate control studies on such individuals revealed a higher mean response than our usual control group. Thus, differences would have been accentuated by the use of strictly age-matched controls.

RESULTS

The first three patients represent a heterogeneous group of severe immune disorders (Table 1). Patient 1, with ADA deficiency, presented with the most immunologically severe SCID, but is fully corrected following bone marrow transplantation. The disorder of patient 2 is characterized by circulating B cells, evidence of specific antibody formation, and minimal residual T-cell function as reflected by the lymphocyte proliferative response to allogeneic cells in the MLC. Erythrocyte adenosine deaminase and nucleoside phosphorylase levels were normal. Induction of T-cell differentiation in vitro with various thymic factors was incomplete, which suggested a defect in the differentiation of stem cells or precursor T cells. This impression was supported by the failure of several thymus transplants to improve immunologic responses. Patient 3 represented the other end of the spectrum with partial deficits of B- and T-cell immunity. The increase in the percentage of E-rosetting cells upon incubation with thymosin and the partial immune reconstitution following thymus transplant suggested thymic hypoplasia/hypofunction rather than a differentiation defect. The fourth patient represents primarily a T-cell deficiency with a maturation defect at the postthymic level. No abnormalities of routine blood coagulation studies were found in any of the children, and no clinical manifestations of hemorrhagic disease were noted.

The generation of MCTF was studied in all four patients during various phases of their disease and treatment. As shown in Table 2, all four patients had a severe deficiency in the generation of MCTF. The percentage of monocytes per cell culture was increased in the patient group (range 38%-85%) as compared to controls (range 11%-20%). Therefore, the generation of MCTF activity was expressed as units per monocyte for both groups (see Materials and Methods). All four patients had markedly reduced generation of MCTF activity to PHA stimulation (Table 2). The two patients with SCID and a probable block or defect in stem cell or precursor T-cell differentiation (patients 1 and 2) also had a severe defect in MCTF generation in response to LPS simulation. Patient 3 with thymic hypofunction and patient 4 with T-cell deficiency generated good MCTF activity in response to LPS stimulation.

These latter two patients were partially reconstituted by a thymus transplant and transfer factor.

---

Table 2. Monocyte Tissue Factor (MCTF) Generation in Response to PHA and LPS in Severe Combined Immunodeficiency Disease

<table>
<thead>
<tr>
<th>Patient Description</th>
<th>Percent Monocytes</th>
<th>Tissue Factor Units* (±1 SEM x 10^-9/Monocyte)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1 — adenosine deaminase deficiency (6)†</td>
<td>82-90‡</td>
<td>PHA 2.1 ± 0.1§ LPS 3.8 ± 0.6†</td>
</tr>
<tr>
<td>Patient 2 — circulating B cells and response to allogeneic cells (3)</td>
<td>35-50</td>
<td>PHA 6.2 ± 1.2§ LPS 11.7 ± 5.2†</td>
</tr>
<tr>
<td>Patient 3 — thymic hypoplasia (1)</td>
<td>38</td>
<td>PHA 0.5 LPS 36.3</td>
</tr>
<tr>
<td>Patient 4 — T-cell deficiency (1)</td>
<td>16</td>
<td>PHA 15.9 LPS 34.6</td>
</tr>
<tr>
<td>Normal controls (16)</td>
<td>9-20</td>
<td>PHA 40.3 ± 9.3 LPS 53.1 ± 4.7</td>
</tr>
</tbody>
</table>

*Values for each experiment represent the mean of duplicate cultures.
†The number of experiments performed on separate days is noted in the parentheses.
‡Range of percentage of monocytes in cell culture.
§p < 0.02.
¶p < 0.00001.
Fig. 1. Mononuclear cell tissue factor generation before and after bone marrow transplantation in a patient with adenosine deaminase deficiency. Each bar represents the mean value of duplicate determination. Control values are plotted as the mean ± 1 SEM (brackets) for 16 cultures.

respectively, at the time of testing. Patient 1, with ADA deficiency, was studied on several occasions before bone marrow transplantation and was found to be consistently abnormal. After bone marrow transplantation, MCTF generation gradually returned to normal (Fig. 1).

The effect of the increased percentage of monocytes on MCTF generation was examined further. As with B-cell function and immunoglobulin synthesis, an abnormality of MCTF generation could have resulted from either a primary defect in cellular generation or a secondary defect (i.e., inhibition or suppression of MCTF activity). To examine these possibilities, coculture experiments were performed. As shown in Table 3 and Fig. 2, coculture of mononuclear cells from patients 1 and 2 with normal control cells resulted in a marked inhibition of MCTF generation in response to both PHA and LPS. Patient 3 was not studied in coculture. Patient 4 showed no inhibition of MCTF generation in coculture. Coculture of normal control cells from unrelated individuals did not suppress MCTF generation. Cell sonicates or supernatants from the stimulated cultures of the cells from patients 1 and 2 were unsuccessful in producing inhibition of MCTF generation. One year after bone marrow transplantation, patient 1 no longer inhibited MCTF generation in coculture (Fig. 3). Loss of inhibitor activity was accompanied by the return of normal MCTF generation.

DISCUSSION

Four patients with severe combined immunodeficiency or T-cell deficiency were studied for the generation of MCTF activity. The absence of MCTF generation in response to both PHA and LPS in patient 1 is most compatible with a primary or secondary defect in monocyte function. The minimal response to LPS by cells from patient 2 (with no response to PHA) may indicate residual monocyte function with an absence of the T-cell influence necessary for optimal LPS-induced MCTF generation. In cells from patients 3 and 4, the LPS response was minimally abnormal. We postulate that this subnormal response to LPS may reflect the lack of optimal T-cell influence, as suggested by the severe impairment of MCTF generation in response to PHA. Thus, the dichotomy in the response to two different mitogens in our patients...
Fig. 3. Mononuclear cell tissue factor generation and inhibitory activity in coculture before and after bone marrow transplantation in a patient with adenosine deaminase deficiency. Following bone marrow transplantation an increase in tissue factor activity (●) was demonstrated, concomitant with immune reconstitution and the disappearance of inhibitory activity (○) in coculture. An apparent inverse relationship was observed between tissue factor activity and the absolute monocyte count (see text).

lends support to the previous findings of two different pathways for MCTF generation in normal cells: (1) monocytes generate MCTF in response to LPS without the help of T cells or T-cell products; (2) monocytes have an absolute requirement for T-cells or T-cell products, however, for the generation of tissue factor in response to PHA.$^{19,20}$ The $T$-dependent regulation of MCTF generation by monocytes in response to PHA may occur by direct cell-cell interaction or by the production of a lymphokine.* The regulation of monocyte activity by lymphocytes or lymphocyte activation products (lymphokines) has been described for a number of monocyte functions, such as macrophage inhibition factor, monocyte chemotactic factors, and macrophage activating factors.$^{11}$ We recognize that a complete definition of the impairment of monocyte interaction in these patients will require further studies of cell subpopulations with reconstitution experiments.

The decrease in MCTF generation could be secondary to inhibitory factors or cells. For example, in certain types of common variable hypogammaglobulinemia, increased T-suppressor cell activity demonstrable by comixing experiments accounts for the decreased B-cell function.$^{26}$ Inhibitory monocytes have been reported in other clinical disorders. Broder and colleagues$^{27}$ demonstrated that circulating monocytes in patients with multiple myeloma suppress immunoglobulin secretion. Monocytes may inhibit T-cell function in Hodgkin's patients by the generation of prostaglandin or prostaglandin-like products. Similar coculture experiments were performed in our patients (Table 3 and Fig. 2). Two functional groups could be ascertained. The coculture of normal cells with the patients who had a block or defect in stem cell differentiation demonstrated 100% suppression of MCTF generation. In contrast, the patient with T-cell deficiency exhibited no suppression of normal cells in the generation of MCTF. The accumulation of toxic products, such as adenosine or deoxyadenosine,$^{29}$ which results from a block in the purine salvage pathway, might have been responsible for the suppression of MCTF activity in patient 1 with ADA deficiency. However, the other patient (patient 2) with normal red cell adenosine deaminase and nucleoside phosphorylase still demonstrated suppression in coculture. The failure of sonicates and supernatants from stimulated cultures of patient cells to suppress normal cells also argues against toxic metabolic products. These studies do not rule out a possible role for toxic products, but do suggest, however, the importance of intact cells for the suppression of MCTF generation.

Both of these patients had a very high percentage of monocytes (50% and 90%), and the suppression appeared to vary inversely with the absolute monocyte count in the patient with ADA deficiency following bone marrow transplantation (Fig. 3). Although Laughter and Twomey$^{30}$ have reported that normal monocytes cultured at an increased density with normal lymphocytes can suppress lymphoproliferation in response to mitogens, control cells cultured at an equivalent increased monocyte concentration (60%–90%) generate normal MCTF activity in response to LPS. Studies by Edwards and Rickles$^{31}$ demonstrated

---

a positive relationship between MCTF generation and the percentage of monocytes in patients with either cancer and/or reactive monocytosis. The impairment of MCTF generation in patients 1 and 2, therefore, may be due to defective cell function and/or the presence of a population of suppressor cells. Cell separation studies will be necessary for the characterization of this defect(s) and for identification of the type (or types) of suppressor cells.

The possibility that monocyte maturation as well as lymphocyte differentiation is affected as part of the immunodeficiency disorder has to be considered, particularly in light of recent evidence linking the development of myeloid cells (monocytes and granulocytes) with lymphocytes via a common stem cell.12 Pahwa et al.9 have described two patients with SCID (including one patient with ADA deficiency) who had significant defects of monocyte chemotaxis that were corrected following bone marrow transplantation. The defective monocyte chemotaxis9 and the impaired generation of MCTF activity in response to both PHA and LPS in our patients (1 and 2) occurred in those patients who had defects or blocks in stem cell or precursor T-cell differentiation. Fischer et al.33 reported that a specific inhibitor of the adenosine deaminase enzyme, erythro 9-(2-hydroxy-3-nonyl) adenine (or EHNA) prevented the maturation of monocytes to macrophages. In our own laboratories, inhibition of adenosine deaminase activity by EHNA prevents MCTF generation by peripheral blood mononuclear cells in response to both PHA and LPS.34 Similarly, Snyderman and coworkers35 were able to inhibit monocyte chemotaxis by incubation with EHNA and adenosine. These studies suggest that an intact purine salvage pathway is necessary for monocyte and lymphocyte function,36 as well as maturation (? differentiation).

Fibrin, the end-product of activated coagulation, can be found in inflammatory lesions in a variety of immune processes, including the renal allograft rejection reaction, rheumatoid arthritis, and immune vasculitis.37 40 Hattler and colleagues have implicated MCTF in the pathogenesis of this fibrin deposition.40 Therefore, MCTF generation, which occurs in monocytes in response to a variety of immunologic stimuli,13,17,20 may be important for the expression of delayed hypersensitivity reactions. Edwards and Rickets41 have reported that systemic anticoagulation inhibits both the development of maximal delayed skin hypersensitivity induration in humans and the generation of MCTF from stimulated mononuclear cells. More recently, Colvin and colleagues have demonstrated, directly, the lack of induration (and microscopic fibrin) in biopsy specimens of skin tests from patients with congenital afibrinogenemia.42 Therefore, fibrin generation seems to be requisite for the development of skin test reactivity (induration). We suggest that MCTF activation at the site of antigen stimulation may be the mechanism by which fibrin generation occurs. We describe the first report of defective MCTF generation in congenital immunodeficiency disease, which strongly supports a link between cell-mediated immune and cell-mediated coagulation reactions.

ACKNOWLEDGMENT

We wish to thank Margaret Van Why for expert technical assistance and Jane Pultinas and Linda Procko for secretarial assistance. We thank Dr. T. Chan for the determination of adenosine deaminase and nucleoside phosphorylase activities in our patients.

REFERENCES

14. Nemerson Y, Pitlick FA: The tissue factor pathway of blood
Impaired mononuclear cell tissue factor generation in patients with immunodeficiency diseases

M Ballow and FR Rickles