A Novel Lymphocyte Differentiating Factor in Serum of Patients With Mycosis Fungoides and Sezary Syndrome

By Bijan Safai, Robert A. Good, Jeremiah J. Twomey, Verna Lewis, and Gideon Goldstein

Sera from 13 patients with mycosis fungoides and 2 with Sezary syndrome were tested for activity that induces lymphocyte differentiation. Induction of Thy-1.2 antigen and surface immunoglobulin were used, respectively, to measure T- and B-cell differentiation. The indicator cells were null lymphocytes from the spleens of congenitally athymic nude mice. Normal serum induced some T-cell but no B-cell differentiation. The T-cell-inducing activity was ascribed to thymic hormone and declined with advancing age. A totally different pattern emerged with patient serum. T-cell-inducing activity was significantly more active than in normal serum ($p < 0.001$). This activity did not decline with advancing age and was not inhibited by a concentration of ubiquitin, which blocks nonspecific $\beta$-adrenergic induction. B-cell-inducing activity was also present. This novel serum factor (or factors) is a potent inducer of T- and B-lymphocyte differentiation and is associated with neoplastic lymphoproliferation of the T-cell series.

MYCOSIS FUNGOIDES (MF) is a lymphoproliferative disorder with prominent cutaneous involvement that occurs most often in elderly males. Central to this disease is a proliferation of lymphocytes with characteristic morphology that bear thymus-derived (T) cell markers. These neoplastic lymphocytes manifest aneuploidy, impaired proliferation, failure to produce lymphokines, and reduced cytotoxic reactivity. Sezary syndrome (SS) can be considered a leukemic variant in which the neoplastic T cells may have some capacity to function as helper cell in immunoglobulin synthesis. The abnormal T-cell proliferation with MF and SS could result from excessive inductive stimulation, an intrinsic derangement of T-cell precursors, or a combination of both. In this study, serum from patients with these syndromes was tested for activity that promotes both T-cell and B-cell differentiation.

MATERIALS AND METHODS

Studies were performed on 13 patients with MF and 2 with SS. Median age was 61 yr, and the range was 35–94 yr. Nine were males; 13 were white and 2 were black. The diagnosis was made by clinical, histologic, and electron microscopic criteria. Nine patients with MF had disease limited to cutaneous plaques; 4 had lymph node involvement as well. The 2 patients with SS had erythroderma and had leukocyte counts of 120,000 and 11,000/cu mm of blood, respectively, with classical Sezary cells on peripheral blood smears. Twelve patients had received electron beam and a few also had topical nitrogen...
mustard therapy prior to study. Control studies were done on 10 patients in each age decade up to age 70 yr and 5 in the eighth age decade who were hospitalized with rheumatic heart or coronary artery disease. Patient and control sera were tested concurrently. Sera were stored prior to study at $-70^\circ C$ or lyophylized.

Sera were first passed through PM30 membranes (Amicon Corp., Lexington, Mass.), which retained molecules >30,000 daltons that were nonspecifically cytotoxic to mouse cells. Serum filtrates were assayed for T-cell-inducing activity by measuring the induction of Thy-1.2 antigen on spleen cells from nude athymic mice bred on a C3H/He background. Thy-1.2 antigen is a genetically determined antigen present on thymus-derived lymphocytes. Indicator cells were recovered in the effluent after nude mouse spleen cells were incubated in equilibrated nylon-wool columns for 45 min, which depleted them of B cells and macrophages. About 5% of the spleen cells were recovered from the columns, 80% of which were null lymphocytes. Induction incubations included $0.5 \times 10^6$ indicator cells suspended in 0.1 ml medium 199 with 5% bovine serum albumin (BSA) plus 0.1 ml of the material under test and 125 $\mu g/ml$ ubiquitin. Ubiquitin is a nonthymic tissue peptide, which at lower concentrations (e.g., 1 ng/ml), induces early T- and B-cell differentiation via $\beta$-adrenergic receptors. At 125 $\mu g/ml$, nonspecific $\beta$-adrenergic induction is inhibited (<2% Thy-1.2 antigen positive cells), which renders indicator cells more sensitive to low concentrations of thymic hormone. Incubations were for 18 hr at 37$^\circ C$ with 5% CO$_2$ in a humidified incubator. Then 0.025 ml antiserum to Thy-1.2 antigen, prepared by immunizing AKR mice with thymocytes from C3H/He mice, and 0.025 ml guinea pig complement (GIBCO, Grand Island, N.Y.) per $0.25 \times 10^6$ incubated cells were added. Paired incubations, with and without complement, were continued for 60 min, after which cell injury was measured using an enzymatic test in which injured cells are solubilized by incubation with protease. In this test, 2.5 mg/ml protease (Sigma Chemical Co.) was added; the incubation was continued for a further 30 min, and residual cells were counted. This cytotoxicity test was selected over dye exclusion or membrane-bound isotope release because of the high level of sensitivity required to detect the minimal cell injury incurred in this system. Induction with serum filtrates was compared with maximum induction by 1 $\mu g/ml$ thymopoietin. Thymopoietin is a purified thymic peptide hormone. This concentration of thymopoietin induced 28%–31% of the indicator cells to express Thy-1.2 antigen. The excellent correlation that exists between thymic hormone concentration added to induction incubations and the percentage of cells induced to express Thy-1.2 antigen ($r = 0.997$) permitted serum values to be expressed as equivalents in inductive activity to known amounts of thymopoietin. When serum activity exceeded that of the thymopoietin standard, it was diluted with medium; earlier studies showed that there is a good correlation between serum dilution and induction activity. A similar relationship existed between dilution of MF serum and induction.

Similar incubations minus ubiquitin were used to assay for B-cell induction. After 18 hr of incubation, cells were washed with phosphate-buffered saline (PBS) and treated for 45 min at 4$^\circ C$ with fluorescein-conjugated antiserum to mouse IgG, IgA, and IgM (Meloy Laboratories, Springfield, Va.). The cells were then washed and studied for membrane fluorescence.

Results on patients and control subjects were compared statistically using the Wilcoxon Rank Sum test. When individual sera were retested for T-cell inductive activity ($n = 10$), reproducibility was within 10%.

**RESULTS**

The shaded areas in Fig. 1 represent 2 SD from the mean of serum T-cell inductive activity on 10 control subjects from each age decade up to age 70 yr. Results were not influenced by sex or race. Serum from control subjects over age 70 yr lacked inductive activity.

Serum from all 15 patients with MF or SS had significantly ($p < 0.001$) elevated T-cell inductive activity (Fig. 1). When serum from four patients was retested, essentially similar results were obtained. Values on serum from patients did not decline with advancing age in contrast to the activity in normal serum. Indeed, the highest activity was recorded on a 94-yr-old male. These results were not influenced by sex, race, whether MF had extended beyond the skin to involve lymph nodes or whether patients had received therapy.

About 80% of spleen cells in effluents from nylon-wool incubations are lympho...
LYMPHOCYTE DIFFERENTIATING FACTOR IN MF AND SS

Fig. 1. Thy-1.2 antigen induction by serum filtrates from normal subjects (shaded areas) and patients (dots) with mycosis fungoides or Sezary syndrome.

cytes. After 18 hr of incubation with medium alone, 10% bore surface Ig and none expressed Thy-1.2 antigen (Table 1). Optimal stimulation with 1 µg/ml thymopoietin induced Thy-1.2 antigen on 30% of indicator cells but did not increase the percentage of cells with membrane immunoglobulin. After incubation with 1 ng/ml ubiquitin, 74% bore either surface immunoglobulin or Thy-1.2 antigen. Serum from three healthy adults in the third age decade induced 12%–19% of indicator cells to express Thy-1.2 but did not increase the concentration of cells bearing membrane immunoglobulin above baseline values. In contrast, undiluted serum from four unselected patients with MF caused significantly higher percentages of cells to bear both T-cell or B-cell markers (p < 0.05). A similar abnormal pattern of induction was recorded with serum from a patient with SS.

DISCUSSION

This article presents evidence that the malignant proliferation of T cells with MF and SS is accompanied by the presence in serum of a factor or factors (MF factor) that initiate brisk differentiation of null lymphocytes. Differentiation was

<table>
<thead>
<tr>
<th>Inducing Substance</th>
<th>Percent Thy-1.2 Antigen Positive Cells</th>
<th>Percent B Cells</th>
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<tbody>
<tr>
<td>Medium blank</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>1 ng/ml Ubiquitin</td>
<td>27</td>
<td>47</td>
</tr>
<tr>
<td>1 µg/ml Thymopoietin</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>Serum from healthy young adults</td>
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<td></td>
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<tr>
<td>Subject I</td>
<td>19</td>
<td>12</td>
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<tr>
<td>Subject II</td>
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<td>12</td>
</tr>
<tr>
<td>Subject III</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Serum from patients with MF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient I</td>
<td>≥30</td>
<td>38</td>
</tr>
<tr>
<td>Patient II</td>
<td>≥30</td>
<td>46</td>
</tr>
<tr>
<td>Patient III</td>
<td>≥30</td>
<td>42</td>
</tr>
<tr>
<td>Patient IV</td>
<td>≥30</td>
<td>40</td>
</tr>
<tr>
<td>Serum from patient with Sezary syndrome</td>
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Table 2. Comparison of Thymopoietin Ubiquitin and MF Factor

<table>
<thead>
<tr>
<th></th>
<th>Thymopoietin</th>
<th>Ubiquitin</th>
<th>MF Factor</th>
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<tbody>
<tr>
<td>Present in normal serum</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Induces Thy-1.2 antigen</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Induces membrane Ig</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Inhibited with 125 μg/ml ubiquitin</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
</tbody>
</table>

At < 250 ng/ml, ubiquitin induces lymphocyte differentiation, but at 125 μg/ml, it inhibits differentiation.12

expressed by the appearance of Thy-1.2 antigen and membrane immunoglobulin on precursor lymphocytes. The higher level of T-cell antigen induction, its persistence at high levels into the tenth age decade, and the induction of B-cell differentiation as well as T-cell differentiation distinguish MF factor from inducing activity in normal serum. The activity in normal serum is restricted to T-cell differentiation, is not inactivated by 125 μg/ml ubiquitin,12 declines with advancing age,20 and is absent in serum from congenital athymic mice,12 patients 1 wk after thymectomy,12 and patients with DiGeorge syndrome.13 This activity was not found in serum from 35 patients with Hodgkin disease, 16 patients with lymphoblastic leukemia, or 5 patients with myeloblastic leukemia. This inductive activity in normal serum detected by the present bioassay is similar to that of the thymic hormone, thymopoietin. The MF factor has obviously different characteristics.

It is apparent from Table 2 that the pattern of inductive activity for MF factor is distinct from that of ubiquitin as well as thymopoietin. All three substances induce brisk T-cell differentiation.15 Both ubiquitin and MF factor induce B-cell differentiation, while thymopoietin does not. High concentrations of ubiquitin are self-inhibitory12 but do not inhibit T-cell induction with MF factor (Fig. 1) as well as thymopoietin.21 Ubiquitin and MF factor are not found in normal serum. Facteur thymique serique is a serum peptide, believed to be of thymic origin21 that differs in amino acid sequence from thymopoietin and ubiquitin.22 Facteur thymique serique also induces both T-cell and B-cell differentiation but can be distinguished from MF factor by the fact that it is inhibited by high concentrations of ubiquitin.22 Thus, MF factor appears to be a previously undescribed substance with unique lymphocyte-differentiating activity found in serum from patients with MF or SS. In studies to be reported later, we have found T-cell-inducing activity to be low in many sera from patients with Hodgkin disease or lymphoblastic leukemia. It remains to be determined whether MF factor is a new, disease-related product or a physiologic substance that is present in undetectable amounts in normal serum.

Most of our patients were in an age range where the thymus normally is involuted and circulating thymic hormone activity is greatly reduced or absent.20,21 There is no evidence of structural abnormality of the thymus with MF, such as persistence of a juvenile architecture. Furthermore, MF factor has lymphocyte-differentiating characteristics that distinguish it from known physiologic thymic products or ubiquitin. Therefore, it is possible that MF factor is produced by nonthymic tissues.

Normally, T-cell precursors migrate to the thymus, in which environment they differentiate under the influence of hormones secreted by thymic epithelial cells.23 With MF, neoplastic T cells are primarily found in the skin and later in lymphoid tissues. Perhaps this represents a homing phenomenon to the skin analogous to normal T-cell homing to the juvenile thymus. Cutaneous epithelial elements of
patients with MF may acquire the capacity for MF factor secretion. It is also possible that MF factor is produced by the malignant cells themselves. If MF factor has an etiologic role in abnormal T-cell proliferation with MF and SS, it is surprising that the proliferative process is limited to the T cell-series, since this substance induced both T- and B-cell differentiation in vitro. It is possible that other microenvironmental factors restrict the proliferative process in vivo to the T-cell series.

REFERENCES

19. Schlesinger DH, Goldstein G: The amino acid sequence of thymopoietin II. Cell 5:361, 1975