The Relationship between Disease Activity, Treatment Response, and Immunologic Reactivity in Immunoblastic Lymphadenopathy:

A Longitudinal Study of Treatment with Levamisole and Cytostatics

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In a longitudinal study, several immunologic *in vitro* tests were performed on peripheral lymphocytes in four patients with immunoblastic lymphadenopathy during sequential therapy with Levamisole and polychemotherapy.

The percentage of T cells tended to fall with increasing disease activity, while the percentage of B cells remained almost constant.

Blast transformation tests showed that both the T-cell and B-cell responses are defective and that improvement in disease status is correlated closely with an improvement of the blast transformation response. Levamisole treatment improved the blast transformation response but did not produce clinical remission in contrast to polychemotherapy, which produced clinical remission as well as improvement of the blast transformation response.

No conclusions about the pathogenesis of this disease could be made in the present study. No proof was found of a primary defective T-cell function (including T-suppressor activity) although the available methods did not preclude this possibility completely.

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THE CLASSIC DESCRIPTION of immunoblastic lymphadenopathy (IL) was given by Frizzera *et al.*[5] and by Lukes and Tindle.[9] As of January 1977, 89 cases had been described in the literature and were reviewed by Neiman *et al.*[11] who supplied a further six cases. The etiology and pathogenesis of IL are both still unknown, and it remains unsettled whether this disease represents a true malignancy or a paramalignant immune reaction. To various investigators, the clinicopathologic picture has suggested a graft-versus-host reaction,[5] an autoimmune reaction,[6] or continual antigenic stimulation,[16] respectively. Lukes *et al.*[9] emphasized a hyperimmune B-cell proliferation, but in several later reports.[1,4,7,8,11,14] a T-cell deficiency has been demonstrated. Some have speculated that the proliferation of B lymphocytes is a secondary phenomenon due to a loss of suppressor T cells[6,8,11] in analogy with the postulated pathogenesis of several clinical and experimental autoimmune disorders. A proof of this theory is still lacking, however.

Only two longitudinal studies have been published of the immune response in IL during treatment.[1,4] In both studies, it was found that a decreased number of T lymphocytes in the peripheral blood could be normalized temporarily by treatment with Levamisole and that this normalization was associated with a decreased disease activity. Simultaneous blast transformation tests were not done, however.

To elucidate the relationships between disease activity, treatment response, and immunologic reactivity in IL, we conducted a longitudinal study in four patients in whom the function of the immune system was monitored by rosette formation tests and blast transformation test of peripheral lymphocytes.

Materials and Methods

Patients

Four patients fulfilling the histopathologic and clinical criteria required for the diagnosis of IL were studied. Table 1 shows the pertinent anamnestic data and clinical findings at diagnosis. Polyclonal hyper-

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gammaglobulinemia was found in all patients except in case 2. However, the diagnosis of IL was confirmed in this patient by repeated lymph node biopsies, all of which showed the typical histopathologic changes.

Patient 1 died of a generalized candidiasis 14 months after diagnosis, but the three remaining patients were alive as of May 1, 1979, from 17–29 months after diagnosis.

The disease activity according to the prominence of both the subjective complaints and objective signs of disease has been indicated in Figures 1–4. In all patients, a complete or near-complete remission was obtained with polychemotherapy, but these remissions were brief, from two to four months. However, in case 2, the brevity of the first remission was probably caused by a too long interval (more than two months) between the two first courses of chemotherapy. With monthly courses of the same chemotherapy, the second remission in this case lasted five months. Figures 1–4 only depict the disease activity during the immunologic measurements, but in all the patients, the subsequent course has been one of constant, modest disease activity, interrupted by frequent exacerbations. With the exception of patient 1 who needed hospitalization during the terminal phase of his illness, the patients were treated on an outpatient basis.

Treatments

During the study period, only two treatments were employed. After a brief initial period of observation to obtain baseline values for the various immunologic tests, treatment was started with the antianergic drug Levamisole in a dose of 2.5 mg/kg for two consecutive days weekly. From two to eight weeks later, this treatment was supplemented with monthly courses of polychemotherapy, since it was considered impossible to continue with Levamisole as the only treatment when pronounced, persistent (case 1, 2, 4), or progressive (case 3) symptoms (Table 1) were present. The polychemotherapy consisted of Adriamycin, 60 mg day 1, cyclophosphamide, 600 mg day 1, vincristine, 2 mg day 1, and prednisolone, 100 mg daily for five days. After the end of the study period, prednisolone has been given during prolonged periods in cases 1 and 3, and alternative polychemotherapy has been given in cases 1 and 2, followed by clinical improvement.

Tests

Blood samples for *in vitro* immunologic tests were obtained once weekly or biweekly in all patients during periods ranging from three to ten months. Leukocyte and differential counts were done simultaneously. Skin tests were not done.

TABLE 1. Summary of Anamnestic and Clinical Data before Treatment in Four Patients with Immunoblastic Lymphadenopathy

	Patient 1	Patient 2	Patient 3	Patient 4
Sex and age at diagnosis	M 60	M 66	F 54	M 56
Symptom duration (mo.)	6	12	1	2
Drug allergy	+	–	+	–
Fever	+	+	+	–
Weight loss	–	+	–	–
Exanthema	+	+	+	+
Pruritus	+	+	+	+
Sweats	+	+	–	+
Lymphadenopathy	+	+	+	+
Hepatomegaly	+	+	+	+
Splenomegaly	+	–	–	+
Hypergammaglobulinemia	IgG IgM	–	IgA IgM	IgG IgA IgM
Anemia	–	+	–	–
Coombs' test+	?	?	–	+
<1000 lymphocytes/mm³	+	+	–	–
Survival (mo.)	14	29+	27+	17+

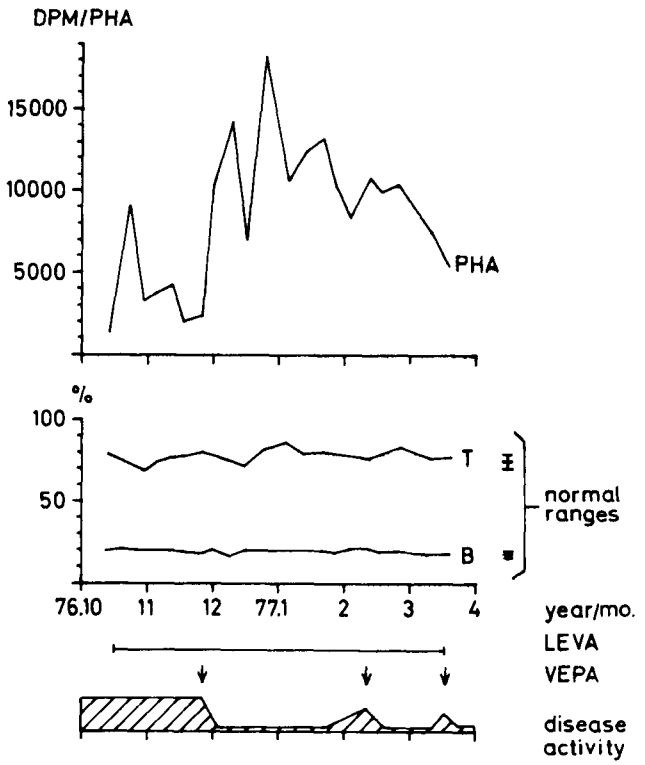

FIG. 1. Case 1: Blast transformation response and percentage of T and B lymphocytes related to treatment and disease activity. The blast transformation response to phytohemagglutinin (PHA) is expressed as disintegrations per minute (DPM). Normal range for PHA: 16851 ± 4094 DPM. LEVA = Levamisole treatment; VEPA = polychemotherapy with vincristine, cyclophosphamide, prednisolone and Adriamycin.

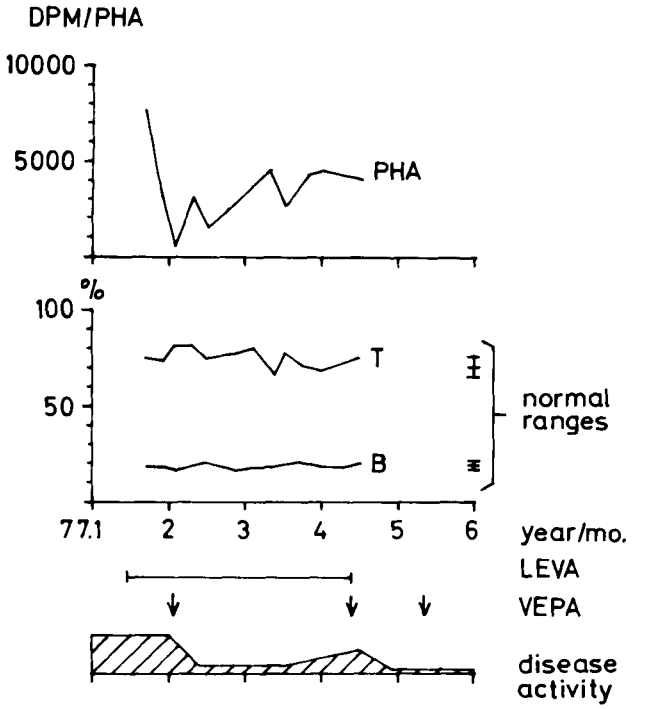

FIG. 2. Case 2: Blast transformation response and percentage of T and B lymphocytes related to treatment and disease activity. The blast transformation response to phytohemagglutinin (PHA) is expressed as disintegrations per minute (DPM). Normal range for PHA: 15,483 ± 3314 DPM.

Lymphocytes were separated from heparinized venous blood and frozen-stored, using a cryobiologic freezing program with Dimethyl Sulfoxide as cryoprotective agent in the freezing medium. The lymphocyte samples were frozen to −95 C and stored at this temperature until a series of patients' samples was complete. All the samples in a series were then thawed and used in a single culture or rosetting experiment to reduce day-to-day variation.

T and B lymphocytes were assayed, using the rosette technique as described previously.[2] The number of B cells was evaluated by testing for HEAC-rosettes, using human A-group erythrocytes, rabbit anti-A, and mouse complement. The number of T cells was found by forming E-rosettes, using unsensitized sheep red blood cells and absorbed human AB-serum. Results are given in percents. Nil cells are non-T and non-B cells.

Blast transformation tests were done using 0.5 ml cultures containing 2×10^5 responder cells and stimulated with phytohemagglutinin (PHA; Difco; 6.25 μg/vial), pokeweed mitogen (PWM; Difco; 500 μg/vial), tuberculin purified protein derivated (PPD; State Serum Institute, Copenhagen; 50,000 IU/ml 0.2 μg/vial), concanavalin A (ConA; Pharmacia; 3.125 μg/vial), *candida albicans* (CA; provided by Dr. E. Svejgaard, MD, Copenhagen, 10 mg protein/ml, diluted 1:1000; 250 μg/vial), *E. coli* (EC) and *staphylococcus aureus* (SA) (provided by Dr. K. Jensen, MD, Copenhagen; 10^9 *S. aureus* or *E. Coli*/ml, diluted 1:100, 250 μl/vial), streptokinase/streptodornase (SK/SD; Lederle; 100,000 IU streptokinase + 25,000 IU streptodornase, diluted with 10 ml sterile water, dialyzed for 24 hours against four changes of 0.9% NaCl, dialyzed for 24 hours against medium TC-199 and sterile filtered; 250 μg/vial), and allogenic lymphocytes (2×10^5 stimulator cells) from a pool of 3 HLA-nonidentical normal donors, irradiated with 20 Gy (MLC). All of the above blast transformation tests were done in cases 3 and 4. Only PHA, PWM, PPD, and MLC were done in cases 1 and 2.

Blast transformation was measured in a liquid scintillation counter after incubation for six days and 2-C^{14} thymidine incorporation for 20 hours. Cultures were carried out in triplicates and results were given in mean ±1 SD as disintegrations per minute (dpm). Details of freezing and culture techniques have been given elsewhere.[3]

Results

Presentation

The relationship between disease activity, treatment response, and immunologic reactivity has been shown in Figures 1–4. In case 2, baseline values were not obtained, and the initial values illustrated in Figure 2 represent the immunologic status one week after the start of Levamisole. In the blast transformation tests, very good correlation was observed between the results obtained with PHA, PWM, PPD, and MLC. The results obtained with the other T- and B-cell stimulators were similar. For the sake of clarity, however, only the results obtained with PHA, ConA and EC are given in the figures. The percentages of T and B lymphocytes are given rather than the absolute numbers, because we do not find the routine differential counts sufficiently accurate for a reliable estimate of absolute lymphocyte numbers. The normal ranges indicated for T and B cells and blast transformation tests are age- and sex-related.

Interpretation

Essentially, identical findings have been made in all four patients. At diagnosis, when the disease activity was most pronounced, the blast transformation response was severely depressed. Treatment with Levamisole promptly improved the blast transformation response, but this improvement was only of a few

weeks' duration in spite of continued treatment, and it was in no case associated with any clinical improvement. Since baseline values were not obtained in case 2, we do not know whether the blast transformation response was improved by the Levamisole treatment in this case, but rapid decrease of the blast transformation response shortly after the start of the Levamisole treatment obviously follows the same pattern as seen in the other three cases. The administration of polychemotherapy introduced a new improvement of the blast transformation response, this time of two to four months' duration, accompanied by an unequivocal clinical remission of similar duration. Subsequent clinical relapse was associated with an obvious decrease of the blast transformation response in cases 1, 3, and 4, while the data are incomplete in case 2, which was followed only three months. In the latter case, the improvement of the blast transformation response following chemotherapy was also less striking than in the other cases.

In spite of the generally decreased blast transformation response, the percentage of T lymphocytes was normal (cases 1–3) or decreased (case 4). The lowest values were, however, associated with periods of the most pronounced disease activity in cases 1, 3, and 4. The Levamisole treatment was not followed by a rise in the percentage of T cells. The absolute number of T lymphocytes was within the normal range in all four patients.

The percentage of B lymphocytes was normal in cases 1–3 but decreased in case 4 in which the percentage of nil-cells was constantly higher than that of the B cells. The percentage of B lymphocytes showed almost no fluctuations during the course of the disease in any of the four patients regardless of clinical disease activity.

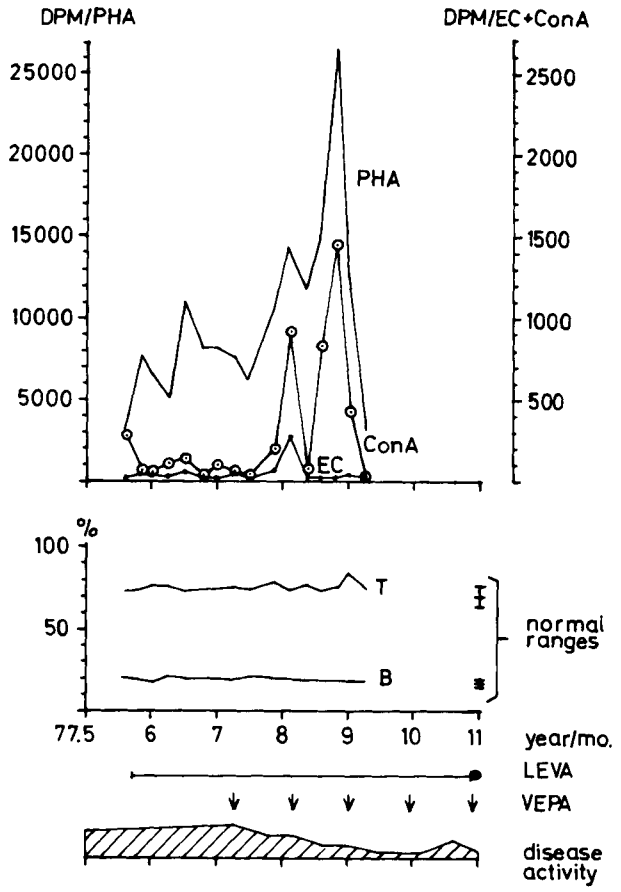

FIG. 3. Case 3: See text to Figure 1. The blast transformation responses to concanavalin A (ConA) and *E. coli.* (EC) are expressed as disintegrations per minute (DPM) on the right vertical scale, while the PHA response is indicated on the left vertical scale as in Figures 1 and 2. Normal range for PHA: 15719 ± 4800 DPM, for ConA: 1060 ± 1405 DPM, and for EC: 129 ± 135 DPM.

Discussion

In the present study, we found that the percentage (or the absolute number) of T cells (or of B cells) is not generally subnormal in IL, but the results of the blast transformation tests show clearly that both the T-cell and B-cell responses are defective. Furthermore, an improvement in disease status is accompanied by an improvement of the T-cell response and possibly also by an increase of the T-cell percentage, as found by Bensa *et al.*[1] and by Ellegaard and Boesen.[4] This pattern does not seem to be reflected in variations in the percentage of B lymphocytes (as found by the above authors), but it is apparently paralleled with the course of the response to the (more or less) B-lymphocyte stimulating agents employed, such as EC.

Levamisole is an antianergic drug, which seems to regulate cell-mediated immune reactions by restoring effector functions of peripheral T lymphocytes and phagocytes and by stimulating precursor T lymphocytes to differentiate into mature cells. There is no concomitant stimulation of the B-cell system.[17] Effector lymphocyte functions, which may be restored, include suppressor activity,[12,15] and suppression of B-cell differentiation by T-cells can be demonstrated already three days after a single dose of levamisole.[13] The fact that there was an unequivocal and instant improvement of the T-cell response after Levamisole treatment without a beneficial clinical effect could mean that a T-cell defect is not an important pathogenetic element in IL. On the other hand, this possibility is not entirely ruled out because the disease at the time of the immunologic evaluation could have passed into a stage where normalization of a pathogenetic factor is not identical with an improvement

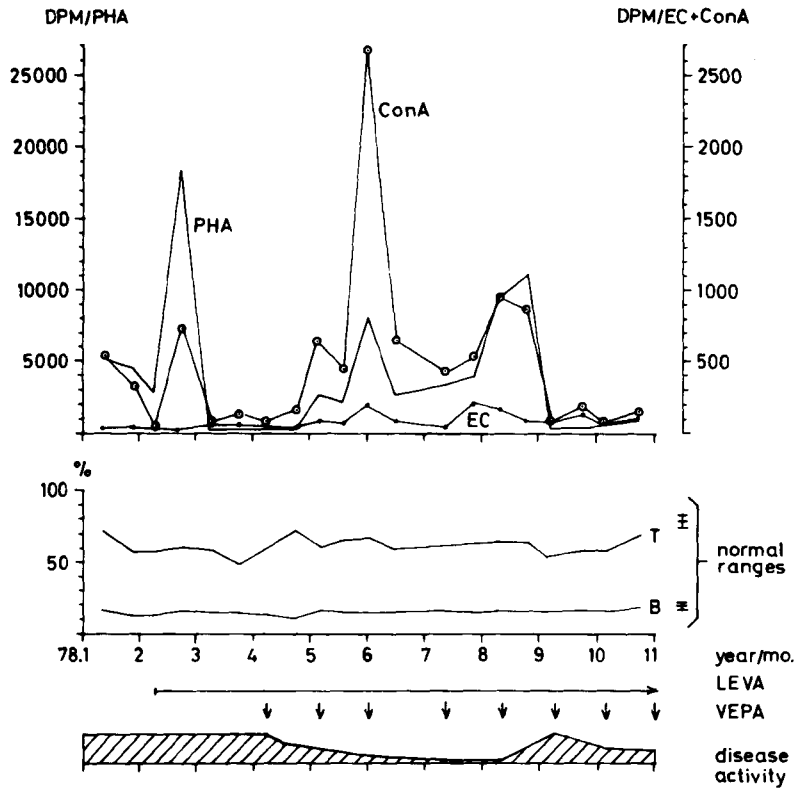

FIG. 4. Case 4. See text to Figure 1 and 3. Normal range for PHA: 17211 ± 2171 DPM, for ConA: 769 ± 621 DPM, and for EC: 530 ± 1033 DPM.

of the disease. As Levamisole is known to restore a defective T-suppressor activity,[12,13,15] the above considerations could also be made about the hypothesis that the proliferation of B lymphocytes is a secondary phenomenon due to a loss of suppressor T cells.[6,8,11]

In the present longitudinal study, the latter theory could be supported if an increased percentage of B cells and an increased B-cell response (as measured by EC stimulation) could be found following a decrease of T-suppressor cell response (as indirectly measured by ConA stimulation). In cases 3 and 4 where all these tests were done, no such correlation could be found, however. There was also no demonstrable time lag between the response of T cells stimulated with ConA and T cells stimulated with PHA or in MLC. There was no difference in the reactions of these cells to treatment with Levamisole. Admittedly, this way of monitoring a suppressor function is rather incomplete, and more firm conclusions have to await the development of more specific tests. Since IL is a disease of the lymphoid tissue, it will always be a question whether the underlying immune abnormalities are reflected by the circulating peripheral lymphocytes. This problem, of course, has to be considered also when interpreting the results of the present study. The fact that polychemotherapy improved the immunologic reactivity as well as the clinical condition of our patients appears to support the rationale of this therapy in IL but does not in itself tell anything about the pathogenesis of the disease.

The lack of clinical effect of Levamisole in all our four patients is at variance with the finding of Bensa *et al.*[1] and Ellegaard and Boesen[4] in the two patients studied by these authors. We are unable to explain this discrepancy satisfactorily, but we are not convinced that Levamisole treatment has a sufficiently rational basis in IL at this moment. If, on the other hand, the available data are taken to mean that Levamisole does modify the course of IL, and if this disease represents a benign condition, it is puzzling why the effect of Levamisole is only transitory. In benign conditions responsive to Levamisole, secondary resistance does not apparently develop. This fact does not prove that IL is a true malignancy, but a recent study indicates that as many as 35% of the cases of IL may terminate as unequivocal malignant lymphomas.[10]

The possibly harmful effect of cytostatics as opposed to corticosteroids in IL has been stressed by several authors.[6,8,18] One of our patients (case 1) died of a generalized candidiasis, which probably developed because of continual prednisolone treatment. Both this patient and the other three patients undoubtedly benefited from cytostatic therapy, and this beneficial effect was at least initially associated with an un-

equivocal improvement of the immunologic reactivity. Although we did not study the immunologic reactivity of our patients during prednisolone treatment for comparison, we feel that our results do justify the use of cytostatic therapy in at least some cases of IL.

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