

Impaired Lymphocyte Transformation in Intestinal Lymphangiectasia: Evidence for at Least Two Functionally Distinct Lymphocyte Populations in Man

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ABSTRACT Intestinal lymphangiectasia is a disease characterized by hypoproteinemia and edema resulting from protein-losing gastroenteropathy secondary to abnormal intestinal lymphatics. Immunologic abnormalities associated with this disease include hypogammaglobulinemia, lymphocytopenia, skin anergy, and impaired allograft rejection. In the present study, the *in vitro* blastogenic transformation of lymphocytes from 12 patients with intestinal lymphangiectasia was assessed in order to gain insight into the mechanism of the cellular immune defect in this disease.

Peripheral blood lymphocytes from patients with intestinal lymphangiectasia showed impaired *in vitro* transformation to nonspecific mitogens, specific antigens, and allogeneic cells when compared to equal numbers of cells from normal individuals. Patients with the most deficient *in vitro* reactivity tended to have the lowest serum albumin concentration and the lowest absolute lymphocyte count. Lymphocytes obtained from chylous effusions in each of the four patients studied transformed more vigorously than peripheral blood cells from the same patients.

These results may be explained by the loss of recirculating, long-lived lymphocytes into the gastrointestinal tract, resulting in a relative depletion of the population of lymphocytes necessary for *in vitro* blast transformation. This disease thus represents a clinical analogue of animals with experimental thoracic duct drainage, and provides evidence for the existence, in man, of two functionally distinct lymphocyte populations. In addition, these findings establish a new mechanism of impaired delayed hypersensitivity and defective *in vitro* lymphocyte transformation, i.e. the gastrointestinal loss and consequent depletion of the long-lived, recirculating population of lymphocytes from the peripheral lymphocyte pool.

INTRODUCTION

Intestinal lymphangiectasia is a chronic congenital or acquired disorder of lymphatics associated with protein-losing gastroenteropathy (1). Patients with this disease have marked hypoproteinemia and edema which is often asymmetrical. The characteristic morphologic lesion of intestinal lymphangiectasia is dilated lymphatic channels in the mucosa of the small bowel. Peripheral lymphatic abnormalities may also be demonstrated by lymphangiography and 45% of the patients develop chylous effusions.

An immune deficiency state with abnormalities of both the humoral and cellular immune systems is associated with intestinal lymphangiectasia (2). Concentrations of IgG, IgA, and IgM are reduced as a result of excessive gastrointestinal protein loss while immunoglobulin synthetic rates are normal. A more marked deficiency of the cellular immune system, as assessed by either intradermal delayed hypersensitivity skin tests or skin allograft survival, is characteristically seen. These findings are associated with a significant peripheral lymphocytopenia which is presumably also due to loss of lymphocyte-rich lymph into the gastrointestinal tract.

The cellular immune deficiency of intestinal lymphangiectasia could be due to the reduced numbers of peripheral blood lymphocytes, in which case one would expect the residual lymphocyte population to function normally *in vitro*. Alternatively, the anergy could re-

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sult from qualitative as well as quantitative changes within the lymphocyte population, such as relative depletion of a necessary subpopulation of lymphocytes, in which case one would expect the residual lymphocyte population to be deficient *in vitro*. We have attempted to distinguish between these alternatives by studying *in vitro* lymphocyte transformation of peripheral blood and chylous fluid lymphocytes to a variety of mitogenic stimuli. These studies provide evidence which strongly favors the second alternative, and support the concept that there are at least two functionally distinct lymphocyte populations in man.

METHODS

Patients studied

Data obtained from 12 patients with intestinal lymphangiectasia are included in this study. All patients had significant protein-losing enteropathy manifested by hypoalbuminemia and edema and documented by excessive fecal loss of albumin-⁵¹Cr (3). In addition, all of the patients had one and usually two or more of the following signs of lymphatic abnormality: lymphocytopenia, dilated lymphatics on intestinal biopsy, abnormal lymphangiogram, chylous effusions, asymmetrical edema, and cutaneous lymphangiomata. In no patient did careful clinical evaluation reveal evidence of any other disorder associated with protein-losing gastroenteropathy (1), and in particular other gastroenteropathies associated with lymphocytopenia (Whipple's disease, regional enteritis, and constrictive pericarditis) were excluded.

The patients ranged in age from 11 to 81 yr; the average duration of disease was 17 yr. They were clinically stable during the periods of study and were free of acute disease, infection, or malignancy. Tests of liver and renal function were generally normal. Associated diseases were limited to one patient each with diabetes mellitus, arteriosclerotic peripheral vascular disease, pulmonary emphysema, and morphea. Drug therapy was limited to diuretics.

Lymphocyte blast transformation *in vitro*

Culture conditions. Lymphocyte transformation was performed as previously described (4) with several modifications. Leukocyte-rich plasma from heparinized peripheral blood was obtained by gravity sedimentation. The number of mononuclear cells was determined by counting in a standard hemocytometer chamber using 0.02% crystal violet in 2% acetic acid as diluent. Duplicate 1 ml cultures containing 5×10^5 mononuclear cells were established in medium RPMI-1640 with 2 mM glutamine, 100 U penicillin, and 100 μ g streptomycin/ml and 10% autologous or homologous plasma. In some early experiments Eagle's minimal essential medium¹ and 20% plasma were used. In most experiments, cells were cultured in autologous plasma and were therefore not washed, but if cells were to be cultured in homologous plasma, they were washed three times at 4°C with plasma-free medium before counting. In multiple control experiments conducted throughout the duration of these studies, no consistent differences in responses were obtained in autologous plasma compared with normal homologous plasma (individual or pooled) and no inhibitor of normal lymphocyte transformation was found in the plasma of patients with

intestinal lymphangiectasia. Therefore, results obtained in all experiments, regardless of medium, are combined in the presentation of data. Cultures were incubated at 37°C in loosely capped flat-bottomed glass vials in a humidified atmosphere of 5% CO₂-95% air for 5 days for nonspecific mitogen and antigen stimulation and 7-8 days for the mixed leukocyte reactions. Control cultures performed simultaneously were established with cells obtained from normal adult volunteer subjects or patients with neurological diseases not affecting the immune system.

Culture stimulants. The following stimulants were used in a total of 1 ml of culture medium: phytohemagglutinin² (PHA),³ 10 μ g; staphylococcal filtrate (SF) prepared according to Ling, Spicer, James, and Williamson (5), 0.1 cc of a 1:10 dilution; pokeweed mitogen (PWM),⁴ 0.1 cc of a 1:10 dilution of the reconstituted powder; streptolysin-O (SLO),⁵ 0.1 ml of a 1:3 dilution of the rehydrated reagent; streptokinase-streptodornase (SKSD),⁶ 110 U SW-27 U SD; diphtheria (Diph) and tetanus (TET) toxoids,⁷ 1 Lf U; *Candida albicans* extract (Cand),⁸ 0.1 ml of a 1:20 dilution of the commercial antigen solution, and vaccinia (Pox),⁹ 0.1 ml of a 1:300 dilution of the 100 dose vial reconstituted with 2 cc medium.

One-way mixed leukocyte cultures (MLC) were performed by culturing leukocyte suspensions containing 2.5×10^6 mononuclear cells with suspensions of irradiated allogeneic leukocytes also containing 2.5×10^6 mononuclear cells. Irradiation of 3000 R was administered at 674 R/min using a 250 KVP Westinghouse¹⁰ Quadrocondex X-ray unit with dual opposed heads separated by 25 cm. This was sufficient irradiation to completely eliminate stimulation of blast transformation in MLC of unrelated subjects when cells from each individual were irradiated, a control included in each experiment.

Harvesting protocol and expression of results

Cell suspensions were pulsed with thymidine-³H for the final 4½ hr of the culture period and the acid precipitable radioactivity determined as described by Oppenheim, Wolstencroft, and Gell (6). Results of duplicate determinations usually agreed within 20% and were averaged. The dpm of the unstimulated culture was then subtracted from the dpm of each stimulated culture to obtain the increment in dpm attributable to the stimulants. Results are expressed as the per cent response of the patient relative to the control individuals' response for each stimulant on each day studied. Six patients were studied on more than one occasion. So

² Burroughs Wellcome & Co. (U. S. A.) Inc., Lot K-9170, Tuckohoe, N. Y.

³ *Abbreviations used in this paper:* Cand, *Candida albicans* extract; Diph, diphtheria toxoid; MLC, mixed leukocyte cultures; PHA, phytohemagglutinin; Pox, vaccinia; PWM, pokeweed mitogen; SF, staphylococcal filtrate; SKSD, streptokinase-streptodornase; SLO, streptolysin-O; TET, tetanus toxoid; WBC, white blood cell.

⁴ Grand Island Biological Co., Grand Island, N. Y.

⁵ Difco Laboratories, Detroit, Mich.

⁶ Varidase, Lederle Laboratories, Pearl River, N. Y. (dialyzed).

⁷ Kindly provided by L. Levin, Massachusetts Department of Health, Boston, Mass.

⁸ Hollister-Stier Laboratories, Inc., Yeadon, Pa. (preservative-free).

⁹ Dryvax, Wyeth Laboratories, Philadelphia, Pa.

¹⁰ Westinghouse Electric Corp., Baltimore, Md.

¹ Microbiological Associates, Inc., Bethesda, Md.

as not to weigh these multiple determinations excessively, the geometric mean of the several determinations of any single stimulant for each patient was calculated and used as the value for this patient.

Lymphocyte counts and serum albumin

Total white blood cell and differential counts were obtained using standard hemotological techniques and the absolute lymphocyte count calculated using the expression, WBC count \times per cent lymphocytes equals absolute lymphocyte count. Serum albumin concentrations were determined either by the bromcresol green method (7) or by electrophoresis. Absolute lymphocyte counts and serum albumin concentrations for each patient represent the mean of at least three determinations during the period studied.

RESULTS

In vitro lymphocyte transformation to nonspecific mitogens. Lymphocytes may be stimulated in vitro to undergo blast transformation by a wide variety of stimulants including some which do not require prior sensitization of the lymphocyte donor (8, 9). These latter stimulants have been termed "nonspecific mitogens." Lymphocytes from patients with intestinal lymphangiectasia show markedly impaired reactivity to three nonspecific mitogens, PHA, SF, and PWM, (Fig. 1), with mean responses of 22-39% of normal. Furthermore, in only one patient was the response to a single nonspecific mitogen normal; all other determinations were less than 66% of normal.

In vitro lymphocyte transformation to specific antigens. Another class of mitogenic stimulants are the specific antigens, which require prior sensitization of the donor with an antigen in order for his lymphocytes to be stimulated to undergo proliferation when exposed to that antigen in vitro (8, 9). As shown in Fig. 2, patients with intestinal lymphangiectasia also show markedly deficient in vitro transformation to all specific antigens. Only 2 of 46 determinations equal or exceed control responses, while 32 are less than 25% of normal. Some caution is necessary in interpreting any individual value, since these results are expressed as a per cent of the response of control individuals and the previous exposure of both patients and controls to any particular antigen is uncertain. Nevertheless, the consistently low responses of the patients with intestinal lymphangiectasia as a group is evident and is not likely to be due solely to chance differences in antigenic exposure.

In vitro lymphocyte transformation to allogeneic cells. A third class of stimulants of in vitro lymphocyte proliferation are allogeneic cells, which are specific stimulants but do not require prior sensitization of the lymphocyte donor to allogeneic antigens (8). Responses to allogeneic cells (i.e. mixed leukocyte cultures) are also significantly impaired in patients with intestinal

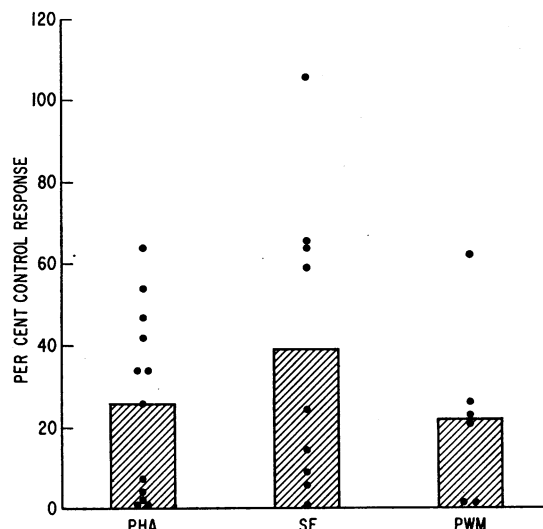


FIGURE 1 In vitro lymphocyte transformation of peripheral blood cells from patients with intestinal lymphangiectasia to nonspecific mitogens. Responses to phytohemagglutinin (PHA), staphylococcal filtrate (SF), and pokeweed mitogen (PWM) for each patient are calculated as described in the text. Bars represent means of values shown.

lymphangiectasia. As may be seen in Fig. 2, the mean response was 27% of normal, and no individual response was greater than 73% of the control. Hence it is apparent that the cells present in the peripheral blood of patients with intestinal lymphangiectasia are deficient in their ability to proliferate in vitro to a wide variety

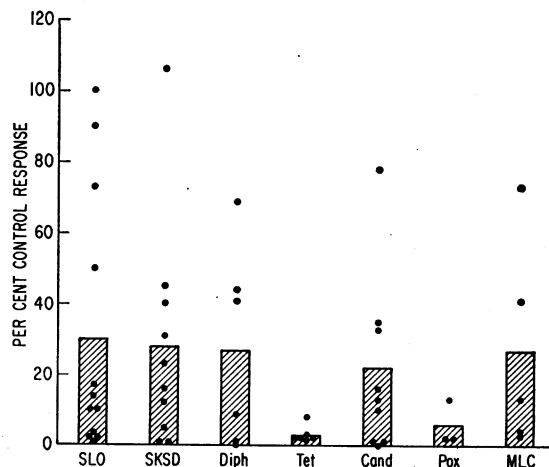


FIGURE 2 In vitro lymphocyte transformation to specific antigens and allogeneic cells. Response to antigens streptolysin-O (SLO), streptokinase-streptodornase (SKSD), diphtheria toxoid (Diph), tetanus toxoid (Tet), Candida (Cand), and vaccinia (Pox), and in mixed leukocyte culture (MLC) are calculated as described in the text. Bars represent means of values shown.

of stimulants, including nonspecific mitogens, specific antigens, and allogeneic cells.

Correlation of lymphocyte transformation with severity of disease. Since patients with intestinal lymphangiectasia vary in the extent of their protein-losing enteropathy and severity of their symptoms, it is of interest to determine whether the degree of impairment of in vitro lymphocyte transformation correlated with other measures of disease activity. The response to the nonspecific mitogen PHA of each patient is plotted against his serum albumin concentration in Fig. 3 and against his absolute peripheral lymphocyte count in Fig. 4. It should be emphasized that the transformation values plotted in these graphs reflect the response to equal numbers of cells, and hence lymphocytopenia per se in any individual patient cannot account for the level of the proliferative response. Impairment of in vitro lymphocyte responsiveness was, in general, greatest in those patients with the lowest serum albumin or lymphocyte levels, and there was a significant positive correlation ($P < 0.05$) between lymphocyte transformation and albumin or lymphocyte levels for the patient group as a whole. This positive correlation suggests that a common factor, such as the degree of gastrointestinal lymphocyte-rich lymph loss, is causally related to the hypoalbuminemia, lymphocytopenia, and impaired in vitro lymphocyte transformation characteristic of intestinal lymphangiectasia.

In vitro transformation of chylous fluid lymphocytes. The presence of chylous effusions in several of these patients with intestinal lymphangiectasia presented the opportunity to study the in vitro transformation of cells from a second body compartment. Fluid was withdrawn from the pleural or peritoneal cavity, the cells washed

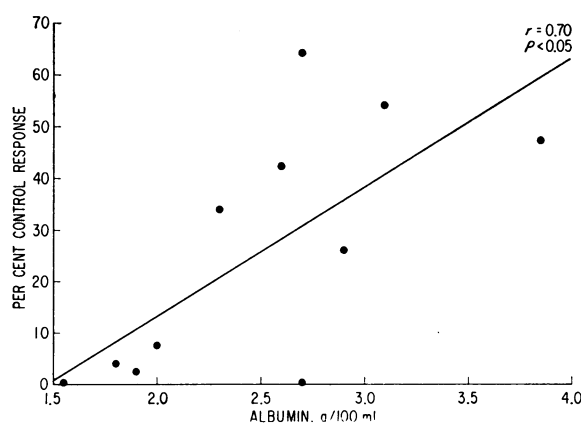


FIGURE 3 In vitro lymphocyte transformation to PHA in each patient studied is plotted vs. his mean serum albumin concentration. The line is drawn by method of least squares; correlation coefficient (r) and level of significance are calculated by standard techniques.

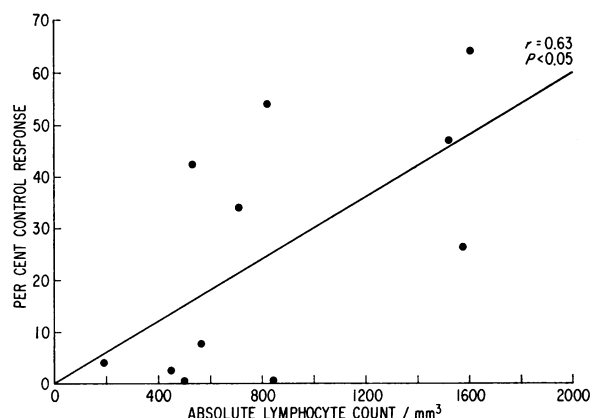


FIGURE 4 In vitro lymphocyte transformation to PHA in each patient studied is plotted vs. his mean absolute peripheral lymphocyte count.

three times in plasma-free medium, and cultured exactly as cells from the peripheral blood. In Fig. 5 the in vitro transformation responses of such chylous fluid cells obtained from four different patients are compared to the responses of cells from the peripheral blood from the same patient. As shown, stimulants used include nonspecific mitogens, antigens, and allogeneic cells. In 17 of 19 pairs, chylous fluid cells were more responsive in vitro than peripheral blood cells, and frequently the response of the patient's chylous fluid cells approached or exceeded that of the control individual's peripheral blood cells. In 15 of the 19 pairs, the chylous fluid cells were 3–100 times more responsive than the corresponding peripheral blood cells, and of the remaining 4 pairs, 2 showed slight increases (+12, +46%) and 2 modest decreases (–11, –24%) in response. These experiments demonstrate that patients with intestinal lymphangiectasia do possess cells capable of responding to mitogenic stimuli, but that these responding cells are relatively depleted in the peripheral blood of such patients.

DISCUSSION

Patients with intestinal lymphangiectasia have been shown previously to have an immunological deficiency state with abnormalities of both the humoral and cellular immune systems (2). Serum immunoglobulin concentrations are reduced in association with normal synthetic rates and increased fractional catabolic rates. The excessive protein catabolism is attributable to the intestinal loss of immunoglobulins along with other serum proteins as demonstrated by studies with albumin- ^{51}Cr (2). Deficiencies within the cellular immune system include depressed skin test reactivity to a battery of delayed hypersensitivity antigens, inability to develop con-

tact sensitivity to dinitrochlorobenzene, and reduced ability to reject skin allografts from unrelated donors.

These immune abnormalities in intestinal lymphangiectasia are associated with lymphocytopenia which appears to be the result of loss of lymphocytes into the gastrointestinal tract. Evidence for this mechanism of lymphocytopenia includes the following: (a) all of the protein-losing gastroenteropathies associated with lymphocytopenia have demonstrable lymphatic abnormalities (10-12), while the nonlymphocytopenic protein-losing gastroenteropathies do not (13-15); (b) in the lymphocytopenic protein-losing gastroenteropathies in which the protein loss is reversible, the lymphocytopenia is also reversible (11, 16-17); (c) lymphatic fluid has been shown to leak directly into the bowel lumen in some patients with intestinal lymphangiectasia, either by aspiration of chyle from the duodenum (18, 19) or by the appearance in the bowel lumen of contrast medium administered during lymphangiography (18, 20); (d) finally, we have demonstrated directly in 2 cases that

lymphocytes of patients with intestinal lymphangiectasia are lost into the gastrointestinal tract. Chylous fluid lymphocytes were washed, labeled with ^{51}Cr , and injected intravenously. Significant radioactivity was detected subsequently in the stool of the patients with intestinal lymphangiectasia but not that of a control individual who also received the labeled lymphocytes.¹¹ Thus it appears that patients with intestinal lymphangiectasia do lose lymphocytes as well as serum proteins into the gastrointestinal tract, most probably as part of the bulk loss of lymph fluid containing both cellular elements and serum proteins.

The in vitro studies of lymphocyte function reported here were undertaken to assess whether the cellular immune defect observed in these patients is simply the result of the reduced number of peripheral blood lymphocytes. We have seen however, that even when cultures were established with equal numbers of mononuclear cells, lymphocytes from patients with intestinal lymphangiectasia still showed impaired in vitro transformation to all mitogens. If the immune deficiency of intestinal lymphangiectasia were solely the result of the quantitative decrease in lymphocyte number, these conditions of in vitro study should correct the deficiency. Since this is not the case, the residual lymphocyte population of patients with intestinal lymphangiectasia must be qualitatively as well as quantitatively defective.

A qualitative defect could occur as a result of the production of defective lymphocytes. However, this possibility is unlikely in view of the fact that in vitro lymphocyte reactivity was roughly correlated with the magnitude of protein loss into the gastrointestinal tract, a process which would not be expected to affect lymphocyte function directly. Furthermore, patients with intestinal lymphangiectasia can produce responsive lymphocytes, as shown by the increased reactivity of the cells in the chylous fluid. A more likely explanation for the qualitative lymphocyte defect lies in the possibility that the peripheral lymphocyte pool in man is a functionally heterogeneous population of cells, and that the intestinal lymphocyte loss in these patients preferentially affects those cells which normally participate in proliferative responses in vitro.

The concept of functional heterogeneity of the peripheral lymphocyte pool is supported by a large body of experimental evidence which indicates that the peripheral blood lymphocyte pool of rodents consists of at least two distinct cell populations (21, 22). One of these is a recirculating population which migrates from the blood through post capillary venules of lymph nodes to reach efferent lymphatics, ultimately returning to the blood via the thoracic duct. This recirculating pool consists primarily of long-lived lymphocytes (life span of months

¹¹ Weiden, P., and W. Strober. Unpublished observations.

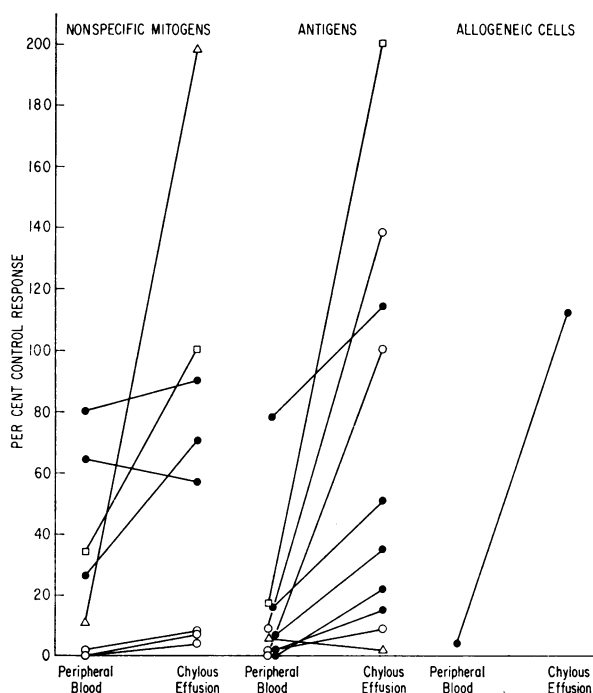


FIGURE 5 In vitro transformation of peripheral blood and chylous effusion lymphocytes in each of four patients (represented by symbols: ●, △, □, ○) to nonspecific mitogens, specific antigens, and allogeneic cells. The values for one patient (●), represent the geometric mean of his responses on 4 different days to the different stimulants within each category; the values of the other patients represent the mean response to each stimulant used within a category obtained in duplicate cultures on a single day. Cells obtained from both pleural (●, ○) and ascitic (△, □) fluid are represented.

to years) which differentiate under the influence of the thymus. A second population of lymphocytes in the peripheral blood consists of short-lived cells (life span of days) which do not recirculate through the lymph. Functional differences between these populations have been demonstrated in various animal systems (23-27). In particular, Iversen (27) has shown that peripheral blood lymphocytes obtained from rats after several days of thoracic duct drainage and hence relatively depleted in recirculating lymphocytes proliferate poorly in vitro to PHA. At the same time thoracic duct lymphocytes, comprised primarily of cells in the recirculating population, respond significantly better. Thus, in the experimental animal, the long-lived, recirculating lymphocyte or its recent progeny appears to be largely responsible for in vitro lymphocyte transformation.

In intestinal lymphangiectasia, the loss of lymphocyte-rich lymph into the gastrointestinal tract would significantly affect only cells which enter the lymph, i.e. the recirculating lymphocyte population, while nonrecirculating cells which are not likely to be present in intestinal lymph would not be affected. The effect of chronic gastrointestinal loss of lymphocytes upon the recirculating population could be profound, especially if this population consists primarily of long-lived cells. For example,¹² if 20% of the recirculating lymphocytes were lost daily

¹² The effect of a bulk loss process on lymphocytes with different life spans can be determined from the following kinetic considerations which have as their basic assumption that the rate of synthesis and delivery of lymphocytes is not altered secondary to the bulk loss process. If lymphocytes are normally randomly eliminated from the recirculating pool, the size of this recirculating pool can be estimated from the equation $P_1 = s_1/k_1$, where P_1 = the number of lymphocytes in the recirculating pool, s_1 = the number of lymphocytes synthesized and delivered into the pool daily, and k_1 is the fractional elimination rate, that is, the fraction of the recirculating pool that is eliminated from the pool daily. This fraction can be determined from: $k_1 = \ln 2/\text{lymphocyte survival half-life in days}$. If a patient had a bulk loss process such as gastrointestinal lymphocyte loss, superimposed on the normal mechanism of lymphocyte turnover, with the fraction of the daily bulk lymphocyte loss defined as k_2 , the new pool size P_2 then becomes: $P_2 = s_2/(k_1 + k_2)$, and the ratio of the lymphocyte pool size of the patient with bulk loss to that of the normal would be

$$\frac{P_2}{P_1} = \frac{\frac{s_2}{k_1 + k_2}}{\frac{s_1}{k_1}} = \frac{s_2}{k_1 + k_2} \cdot \frac{k_1}{s_1}$$

If the rate of lymphocyte synthesis, s , is not altered by the intestinal loss process, then P_2/P_1 is equal to $k_1/(k_1 + k_2)$. Similarly, by somewhat more complex calculations, the effect of a bulk loss process on lymphocytes with a finite life span can be determined, and the value for the P_2/P_1 ratios are quite comparable to those obtained by calculations based on random lymphocyte survival.

while their rate of production remained unchanged, a population with a survival half-life of 100 days would be reduced to 3.4% of its initial number, i.e.

$$\frac{P_2}{P_1} = \frac{k_1}{k_1 + k_2} = \frac{0.693}{100} / \left(\frac{0.693}{100} + 0.2 \right) = 0.034.$$

Similarly, a population with a half-life of 1000 days would be reduced to 0.3% of its initial number. On the other hand a recirculating population which included primarily short-lived cells with a half-life of 1 day would only be reduced to 78% of its initial value by an identical (20%/day) rate of loss.

With a 20% daily loss of lymphocytes superimposed onto a population initially composed of 80% long-lived cells with a survival half-life of 1000 days and 20% cells with a survival half-life of 10 days, the final ratio of long-lived to short-lived cells would drop from the initial value of 4:1 to 1:21, representing a reduction from 80 to 4.8% of the total lymphocyte pool.

From these considerations it is apparent that both the pathway of recirculation and the life span of the recirculating, long-lived lymphocyte population make this population particularly susceptible to the effects of chronic gastrointestinal lymph loss, and result in its relative depletion from the peripheral blood. Therefore, the defective in vitro transformation of peripheral blood lymphocytes in patients with intestinal lymphangiectasia can be most easily explained by depletion of the long-lived, recirculating population of lymphocytes, and at the same time, the more normal proliferative response of cells obtained from chylous effusions can be explained by the relative enrichment of this cell population with recirculating cells which enter the effusion directly with the chylous intestinal lymph. Thus, intestinal lymphangiectasia represents an apparent clinical analogue of the rodents with prolonged thoracic duct drainage, and provides evidence that, just as in rodents, the peripheral lymphocyte pool of man consists of at least two functionally distinct populations.

Deficient in vitro lymphocyte blast transformation has been described in many other diseases characterized by impaired delayed hypersensitivity and clinical anergy. Mechanisms responsible for deficient in vitro transformation in these diseases include abnormal stem-cell development as in Swiss-type agammaglobulinemia (28) and an absence of a normal thymus as in the DiGeorge syndrome (29, 30). Intestinal lymphangiectasia is unlike these or other developmental immune deficiency states, however, in that the immune deficiency characteristic of this disease results from shortened survival rather than from defective production of effector components. Structural lymphatic abnormalities in the mucosa of the small bowel resulting in the loss of lymphocyte-rich lymph into the gastrointestinal tract appear to be re-

sponsible for the short survival of both immunoglobulins and lymphocytes. Patients with intestinal lymphangiectasia thus represent a new mechanism of defective in vitro lymphocyte blast transformation: the loss of the long-lived, recirculating lymphocyte population which is apparently necessary for normal in vitro lymphocyte proliferative responses.

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