JCI The Journal of Clinical Investigation

# The Sézary syndrome: a malignant proliferation of helper T cells.

S Broder, … , B D Meade, T A Waldmann

*J Clin Invest.* 1976;58(6):1297-1306. https://doi.org/10.1172/JCI108585.

Research Article

The Sézary syndrome is a frequently lethal disease characterized by circulating malignant cells of thymus-derived (T)-cell origin. The capacity of circulating malignant lymphocytes from patients with this syndrome to synthesize immunoglobulins and to function as helper or suppressor cells regulating immunoglobulin synthesis by bone marrow-derived (B) lymphocytes was determined. Peripheral blood lymphocytes from normal individuals had geometric mean immunoglobulin synthetic rates of 4,910 ng for IgM, 1,270 ng for IgA, and 1,625 ng for IgG per 2 X 10(6) cells in culture with pokeweed mitogen for 7 days. Purified normal B cells had geometric mean synthetic rates of 198 ng for IgM, 145 ng for IgA, and 102 ng for IgG. Leukemic cells from patients with the Sézary syndrome produced essentially no immunoglobulins. Adding normal T cells to normal B cells restored their immunoglobin producing capacity. Leukemic cells from four of five patients tested had a similar capacity to help immunoglobulin synthesis by purified normal B cells. Additionally, Sézary cells from one patient studied induced a nearly 10-fold increase in IgA synthesis by lymphocytes from a child with ataxia telangiectasia and selective IgA deficiency. Furthermore, these Sézary cells induced more than a 500-fold increase in IgG and IgA synthesis by lymphocytes from a child with Nezelof's syndrome. When Sézary cells were added to normal unfractionated lymphocytes, they did not suppress […]

# The Sézary Syndrome

## A MALIGNANT PROLIFERATION OF HELPER T CELLS

SAMUEL BRODER, RICHARD L. EDELSON, MARVIN A. LUTZNER, DAVID L. NELSON, RICHARD P. MACDERMOTT, MARY E. DURM, CAROLYN K. GOLDMAN, BRUCE D. MEADE, and THOMAS A. WALDMANN

*From the Metabolism and Dermatology Branches, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014, the Department of Dermatology, Columbia College of Physicians and Surgeons, New York 10032, and the Walter Reed Army Hospital, Washington, D.C. 20012*

ABSTRACT The Sézary syndrome is a frequently lethal disease characterized by circulating malignant cells of thymus-derived (T)-cell origin. The capacity of circulating malignant lymphocytes from patients with this syndrome to synthesize immunoglobulins and to function as helper or suppressor cells regulating immunoglobulin synthesis by bone marrow-derived (B) lymphocytes was determined. Peripheral blood lymphocytes from normal individuals had geometric mean immunoglobulin synthetic rates of 4,910 ng for IgM, 1,270 ng for IgA, and 1,625 ng for IgG per $2 \times 10^6$ cells in culture with pokeweed mitogen for 7 days. Purified normal B cells had geometric mean synthetic rates of 198 ng for IgM, 145 ng for IgA, and 102 ng for IgG. Leukemic cells from patients with the Sézary syndrome produced essentially no immunoglobulins. Adding normal T cells to normal B cells restored their immunoglobulin producing capacity. Leukemic cells from four of five patients tested had a similar capacity to help immunoglobulin synthesis by purified normal B cells. Additionally, Sézary cells from one patient studied induced a nearly 10-fold increase in IgA synthesis by lymphocytes from a child with ataxia telangiectasia and selective IgA deficiency. Furthermore, these Sézary cells induced more than a 500-fold increase in IgG and IgA synthesis by lymphocytes from a child with Nezelof's syndrome. When Sézary cells were added to normal unfractionated lymphocytes, they did not suppress immunoglobulin biosynthesis. In addition, unlike the situation observed when large numbers of normal T cells were added to purified B cells, there was no depression of immunoglobulin synthesis at very high malignant T-cell to B-cell ratios. These data support the view that Sézary T cells do not express suppressor cell activity. The results presented in this paper suggest that neoplastic lymphocytes from the majority of patients with the Sézary syndrome originate from a subset of T cells programmed exclusively for helper-like interactions with B cells in their production of immunoglobulin molecules.

*Received for publication 14 July 1975 and in revised form 2 August 1976.*

## INTRODUCTION

The Sézary syndrome is of great clinical and theoretical interest. The hallmarks of this grave disorder are exfoliative erythroderma, generalized lymphadenopathy, and circulating malignant lymphocytes with a propensity to infiltrate skin (1, 2). These circulating neoplastic lymphocytes, referred to as Sézary cells, have a deeply-folded or cerebriform nucleus as their main morphologic feature.

Lymphocytes may be divided into two main categories: bone marrow-derived cells (B cells) and thymus-derived cells (T cells). Furthermore, certain lymphoproliferative diseases can be classified as malignancies of either B-cell or T-cell origin (3). B cells are the immediate precursors of antibody secreting cells. They are characterized by the presence of surface membrane-bound immunoglobulin molecules (4, 5) and receptor for antigen-antibody complement complexes (6) or heat aggregated IgG (7). With these criteria, most cases of chronic lymphocytic leukemia are examples of a B-cell malignancy (8–10). Human T cells may be identified by the spontaneous formation of rosettes with sheep erythrocytes (11, 12) or by lysis in the presence of specific heterologous cytotoxic antisera raised against thymic lymphocyte antigen and rendered specific by prior absorption with B cells (13). With these criteria, Sézary cells from most patients have a T-cell origin (1, 2, 14).

TABLE I
*Summary of Sézary Syndrome Patients Used in Lymphocyte Immunoglobin Production Experiments in Vitro*

| Patient | Age | Sex | Total leukocyte count per cubic millimeter | Percentage of Sézary cells* | Clinical findings |
|---|---|---|---|---|---|
| | *yr* | | | | |
| F. S. | 69 | F | 240,000 | >99 | Exfoliative erythroderma and hepatosplenomegaly |
| J. N. | 52 | M | 157,000 | 98 | Exfoliative erythroderma |
| L. H. | 69 | M | 37,000 | 95 | Exfoliative erythroderma, hepatosplenomegaly, and Sézary cell leukemic infiltration of lungs |
| R. B. | 57 | M | 11,900 | 41 | Exfoliative erythroderma |
| E. H. | 64 | M | 80,100 | 88 | Exfoliative erythroderma, multiple tumor-involved skin plaques, and hepatosplenomegaly |
| M. C. | 56 | F | 140,000 | 95 | Exfoliative erythroderma and hepatosplenomegaly |
| J. H. | 41 | M | 7,300 | 98 | Exfoliative erythroderma, hepatosplenomegaly, and Sézary cell leukemic infiltration of kidneys |

* Expressed as a percentage of total leukocyte count. In all cases, neoplastic Sézary lymphocytes comprised at least 95% of lymphocytes seen on peripheral blood smear.

T cells play a role in a number of immune systems. These cells are critically involved in cell-mediated immunity, which includes such phenomena as delayed hypersensitivity, allograft rejection, and graft vs. host reactions. Normal T cells proliferate when stimulated by certain plant mitogens, such as phytohemagglutinin and concanavalin A (15). They mediate the mixed lymphocyte reaction (16), act as killer cells in models of lymphocyte-induced cytotoxicity (17), and generate certain soluble effector products known as lymphokines (18). In addition, T cells play a critical role in the regulation of humoral immune responses by acting as potentiators (helper cells) or inhibitors (suppressor cells) of the transition of B cells into immunoglobulin producing plasma cells (19, 20). Both antigen-specific and nonspecific helper and suppressor functions have been identified.

The leukemic T cells of patients with the Sézary syndrome have been examined for their capacity to mediate certain of the normal T-cell functions involved in cell-mediated immunity. The overall conclusion is that the Sézary cells from various individuals differ in the degree of normal T-cell activity they can express (1, 2). Thus, the leukemic cells of some patients responded normally to plant mitogens, while the cells of the majority of patients responded poorly or not at all. The Sézary cells from most of the patients did not transform normally to preformed blastogenic factor (2). Leukemic cells from a few patients functioned as both effective stimulators and responders in mixed lymphocyte reactions, whereas cells from others were either weak stimulators, weak responders, or both (1, 2). Sézary cells from most patients were poor killers in assays of T-cell-mediated cytotoxicity (21). On the other hand, the majority of Sézary syndrome patients had leukemic cells that produced a lymphokine resembling migration inhibition factor (22).

The capacity of Sézary cells to act as helper cells or suppressor cells in humoral immune responses has not been investigated. The purpose of this study was to determine whether Sézary cells can regulate the in vitro synthesis of immunoglobulins by lymphocytes derived from normal individuals and from patients with thymic deficiency states. The results indicate that the neoplastic lymphocytes from most of the patients with the Sézary syndrome studied represent a proliferation of T cells that act as helper T cells but not suppressor T cells, in the process of immunoglobulin production.

## METHODS

*Patient population.* Seven patients with the Sézary syndrome undergoing diagnostic evaluation at the National Cancer Institute were studied. All patients in this study had exfoliative erythroderma, generalized lymphadenopathy, and a leukemia of lymphocytes with the membrane properties of T cells and with the characteristic morphologic features of Sézary cells as determined by light or electron microscopy (1, 2). These cells had a high nuclear: cytoplasmic ratio and had deeply-folded, cerebriform nuclei. The absolute abnormal lymphocyte count ranged from 4,900 cells/$mm^3$ to 240,000 cells/$mm^3$ at the time of study (Table I). In all cases, Sézary cells comprised more than 95% of the lymphocytes seen on peripheral blood smear at the time of study. Controls for this study consisted of 22 healthy individuals whose ages ranged from 18 to 56 yr.

*Measurement of immunoglobulin synthesis by lymphocytes in vitro.* To study the transition of circulating lymphocytes into immunoglobulin secreting plasma cells, peripheral blood lymphocytes were cultured in the presence of pokeweed mitogen. This plant lectin is known to induce polyclonal immunoglobulin production in vitro (23, 24). This technique is described in detail elsewhere (24). Briefly, lymphocytes were obtained from 35–50 ml of heparinized, venous blood and washed 12 times with Mishell-Dutton balanced salt solution (National Institutes of Health Media Section, Bethesda, Md.) containing 5% heat-inactivated fetal calf serum (Grand Island Biological Co., Grand Island, N. Y., control C742923) to remove detectable human serum proteins. 2 million lymphocytes were incubated in loosely capped 1-dram vials with pokeweed mitogen (Grand Island Biological Co., lot A232502) at 37°C in 5% $CO_2$ for 7 days in 1-ml RPMI 1640 media (Grand Island Biological Co.) containing 4 mM L-glutamine, 50 U/ml penicillin, 50 $\mu$g/ml streptomycin, and 10% heat-inactivated fetal calf serum. The dose of the pokeweed mitogen was 10$\mu$l/culture, a dose determined to be optimal in preliminary cultures. At the termination of the culture period, the amount of IgM, IgA, and IgG synthesized and secreted into the medium was determined by double-antibody radioimmunoassay of these immunoglobulins with techniques essentially identical to those previously described for IgE (25).

*Preparation of normal T-cell and B-cell populations.* We prepared T cells and populations of B cells freed of T cells by a two-step procedure that takes advantage of the observations that normal human T cells pass through anti-Fab immunoabsorbent columns and form spontaneous rosettes with sheep erythrocytes, whereas normal B cells are selectively retained by immunoabsorbent columns because of their surface-bound immunoglobulin moieties and do not form spontaneous rosettes with sheep erythrocytes. The technique of immunoabsorbent chromatography, which has been described in detail previously (26), was modified slightly in the current study. The mononuclear cells from 250 ml of heparinized whole peripheral blood were isolated by Ficoll-Hypaque centrifugation and washed three times with Hanks' balanced salt solution containing 5% heat-inactivated fetal calf serum. The cells were incubated at 37°C for 30 min. Then, $3 \times 10^8$ cells suspended in RPMI 1640 media, containing 2.5 mM EDTA, were applied to a disposable syringe column which had been packed with 15 ml of Sephadex G-200 beads (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) conjugated to pure rabbit antihuman Fab. The column had been previously equilibrated with RPMI 1640-EDTA buffer. The T-cell population was harvested by elution with 15 ml of buffer and held overnight at 4°C. The column was then washed with an additional 90 ml of buffer. The B-cell fraction was then harvested by competitive elution using RPMI-EDTA buffer containing 10 mg/ml of human gamma globulin (Cohn's Fraction II, Miles Laboratories, Inc., Elkhart, Ind.). The B-cell fraction eluted from the immunoabsorbent column contained B cells, monocytes, and a few T cells. The contaminating T cells were removed by a sheep erythrocyte rosetting technique. The cells eluted from the column were washed twice in RPMI 1640 media containing 4 mM L-glutamine and resuspended to $3 \times 10^6$/ml. Two parts of the washed B-cell fraction were added to one part of heat-inactivated fetal calf serum previously absorbed with sheep erythrocytes, and three parts of a washed sheep erythrocyte suspension containing $10^8$ erythrocytes/ml. The cells were mixed and centrifuged immediately at 1,000 rpm for 5 min and then stored at 4°C overnight. 16 h later the pellet was resuspended, layered over Ficoll-Hypaque, and centrifuged at 1,300 rpm for 40 min. Cells remaining at the interface were termed the B-cell population. An additional fractionation to remove monocytes was not performed. The T-cell and B-cell populations were then processed for in vitro immunoglobulin biosynthesis as described above for unseparated lymphocytes.

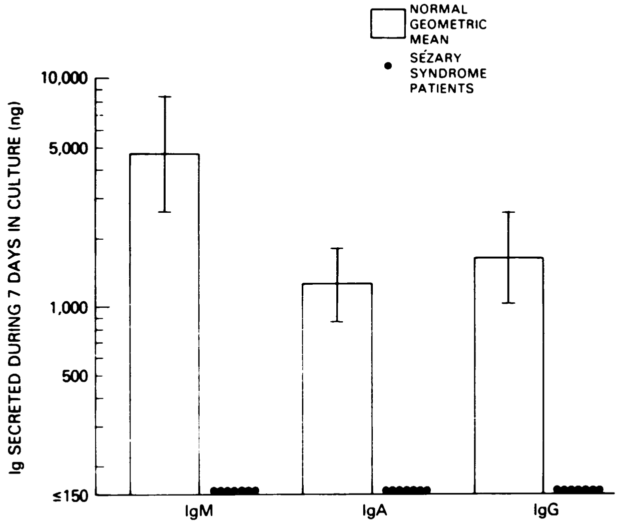


FIGURE 1 Immunoglobulin production by peripheral blood lymphocytes after stimulation by pokeweed mitogen. The geometric means±SD of 22 normal persons and the individual values of 7 patients with the Sézary syndrome are shown.

*Assays of suppressor cell activity.* The presence of circulating suppressor cells was assessed by a co-culture technique (24). Peripheral blood Sézary cells from patients and peripheral blood lymphocytes from controls were cultured together ($1 \times 10^6$ cells from each source) in 1 ml of medium in the presence of pokeweed mitogen. The synthesis of immunoglobulin (Ig) by cells of two subjects in co-culture was related to the sum of the expected contribution by each cell population as follows: Synthesis of Ig by cells in co-culture as percentage of Ig synthesized individually = 100 × Synthesis of Ig by $10^6$ cells from both subjects/½ × sum of Ig synthesized by $2 \times 10^6$ cells of each subject cultured separately.

*Assays of helper cell activity.* As indicated below, pokeweed mitogen-stimulated B-cell populations, freed of T cells, synthesized very small quantities of immunoglobulin molecules. The ability of added cells to augment immunoglobulin synthesis by these B cells was used as a test for helper cell activity. The standard tests for helper activity consisted of culturing $0.5 \times 10^6$ B cells with $1.0 \times 10^6$ purified T cells or Sézary cells in 1 ml of media in the presence of pokeweed mitogen. In certain experiments, where the object was to test the effect of high T-cell to B-cell ratios, $0.2 \times 10^6$ B cells were cultured with varying numbers (from $4 \times 10^5$ to $1.6 \times 10^6$) of normal T cells or Sézary cells.

## RESULTS

*Failure of Sézary cells to produce Ig after stimulation by pokeweed mitogen.* The unseparated peripheral blood lymphocytes from patients with the Sézary syndrome studied had a markedly reduced capacity to produce immunoglobulins in vitro. As shown in Fig. 1,

TABLE II

*Failure of Sézary Cells to Inhibit Ig Synthesis When Co-cultured with Normal Peripheral Blood Lymphocytes. Ig Synthesis by Normal Lymphocytes Co-cultured with Sézary Cells Expressed as a Percentage of Expected Synthesis for Normal Lymphocytes Cultured Alone*

| Patient | IgM | IgA | IgG |
|---|---|---|---|
| F. S. | 320 | 410 | 470 |
| J. N. | 170 | 171 | 143 |
| R. B. | 115 | 121 | 87 |
| E. H. | 155 | 191 | 109 |
| M. C. | Not done | 104 | 81 |

whereas the peripheral blood lymphocytes of 22 normal individuals had geometric mean synthetic rates of 4,910 ng for IgM, 1,270 ng for IgA, and 1,625 ng for IgG, the peripheral blood lymphocytes from the seven Sézary syndrome patients studied produced essentially no immunoglobulins. This observation is in accord with the view that the Sézary leukemic cells are T cells and thus lack the capacity to become immunoglobulin synthesizing and secreting cells. The low immunoglobulin synthesis by the cells of the patients with the Sézary syndrome studied probably reflects the dilution of the B cells of these patients by leukemic T cells. Thus, very few B cells were present in the 2 million lymphocytes cultured from these patients. The alternative possibility that the Sézary cells suppressed the transition of B cells into plasma cells was excluded by the studies presented below.

*Absence of suppressor cell activity by Sézary cells.* It is known that peripheral blood mononuclear cell populations from certain immunodeficient patients contain suppressor cells (24, 27). The peripheral blood T cells from such patients depressed the in vitro synthesis of immunoglobulins by co-cultured normal lymphocytes by 85–100%. Since circulating lymphocytes from Sézary syndrome patients produced very small quantities of immunoglobulins, we tested whether such cells could suppress the Ig synthesis of co-cultured lymphocytes from normal individuals. As shown in Table II, $1 \times 10^6$ circulating lymphocytes from the five Sézary syndrome patients tested failed to inhibit immunoglobulin synthesis when co-cultured with equal numbers of normal peripheral blood lymphocytes. This provides evidence that circulating lymphocytes from patients with the Sézary syndrome do not have detectable suppressor cell activity. Further evidence that Sézary cells lack even the modest suppressor cell activity of normal peripheral blood polyclonal T cells is presented below.

*Helper activity of normal T cells for immunoglobulin synthesis.* We next turned our attention to the capacity of T cells to function as helper cells that augment the transition of normal B cells into immunoglobulin synthesizing and secreting cells. The data based on seven normal individuals is summarized in Fig. 2. Pokeweed mitogen-stimulated normal B cells freed of T cells ($0.5 \times 10^6$ cells/culture) had very low synthetic rates for IgM, IgA, and IgG, with geometric synthetic rates of 198 ng for IgM, 145 ng for IgA, and 102 ng for IgG. In addition, purified T cells ($10^6$ cells) from normal individuals did not produce detectable immunoglobulins when cultured in vitro with pokeweed mitogen. However, the addition of $10^6$ normal T cells to $0.5 \times 10^6$ normal B cells restored the capacity of these B cells to produce large quantities of all three major immunoglobulin classes with geometric synthetic rates of 1,820 ng for IgM, 1,071 ng for IgA, and 1,122 ng for IgG. Other workers have also shown that immunoglobulin synthesis by human peripheral blood lymphocytes stimulated with pokeweed mitogen required helper T cells (28, 29). Helper activity could

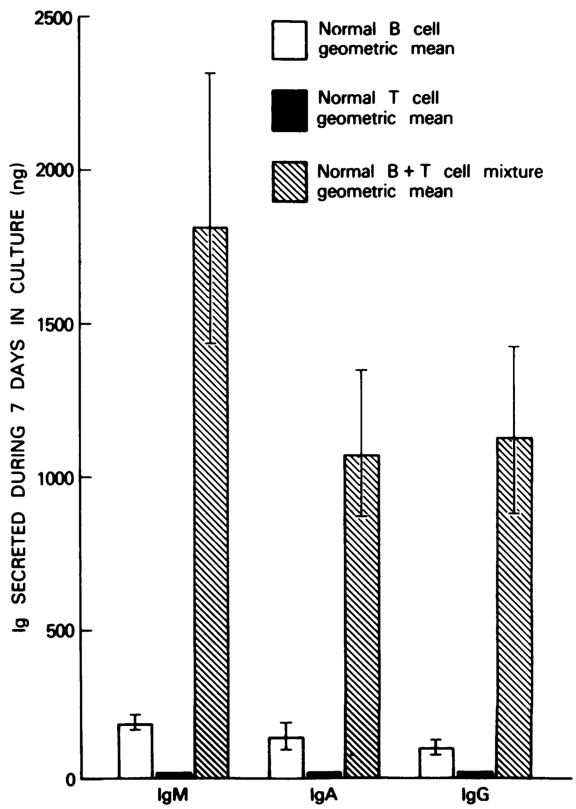


FIGURE 2 Immunoglobulin production by normal B cells alone, normal T cells alone, and mixtures of B cells plus T cells. The geometric means±SD of seven normal persons are shown. Note the drastic impairment of immunoglobulin secretion by purified B cells. Immunoglobulin production is restored (i.e., there is a helper effect) by the addition of T cells. In these experiments, $0.5 \times 10^6$ B cells were co-cultured with $1.0 \times 10^6$ T cells.

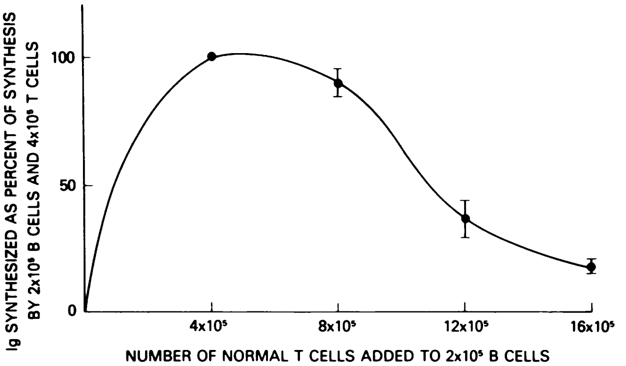


FIGURE 3 Immunoglobulin production by normal B cells as a function of the number of normal T cells added to the culture. The vertical bars represent the SD of the mean for each point. Immunoglobulin (Ig) values illustrated represent IgA production. Note that at high normal T-cell to B-cell ratios immunoglobulin production declines.

be seen when even small numbers of normal T cells were added. Thus, as few as 10,000 T cells produced significant augmentation of immunoglobulin synthesis when they were added to 500,000 normal B cells. When progressively increasing numbers of normal, polyclonal T cells ($4 \times 10^5$–$1.6 \times 10^6$ cells) were added to a constant number of B cells ($2 \times 10^5$ cells), an interesting pattern was observed (Fig. 3). When small numbers of T cells were added to the B cells, an augmentation of immunoglobulin synthesis was observed. However, when the ratio of T cells added to the constant number of B cells exceeded four:one, there was a progressive suppression of immunoglobulin synthesis until, at a ratio of eight T cells to one B cell, the synthesis of immunoglobulin was less than 30% of the peak level. These observations are consistent with the view that normal peripheral blood T cells include both helper and suppressor T-cell subpopulations.

*Helper activity of Sézary cells.* We then tested the capacity of the leukemic lymphocytes from patients with the Sézary syndrome to function as cells that help normal B cells to produce immunoglobulins. Four of the five patients tested had peripheral blood neoplastic cells which retained helper activity. The helper activity of the Sézary cells from these four patients is demonstrated in Fig. 4. There was a 6- to over 30-fold augmentation of immunoglobulin synthesis by B cells after the addition of Sézary cells. A series of control studies was initiated to define the significance of this helper cell activity of the Sézary cells. The first control studies were designed to determine if the helper cell function was a specific property of the cells from the patients with the Sézary syndrome or was merely the result of co-culturing allogeneic cells. In these studies, it was found that the culturing of lymphocytes from patients with chronic lymphocytic leukemia (a malignancy of B cells) or cells of the long-term B-cell tissue culture lines NC 37 and PA3 did not augment immunoglobulin synthesis by purified normal B cells. Similarly, the long-term T-cell line, MOLT IV, did not mediate helper activity. This supports the view that the mere co-culture of allogeneic cells does not lead to apparent helper cell activity. A second series of control studies was designed to exclude the possibility that the helper cell activity of the peripheral blood Sézary cells was due to a small number of contaminating normal T cells. As shown in Fig. 5, some helper activity was observed when as few as 10,000 Sézary cells were added to 500,000 normal B cells. At this dilution, the Sézary cells were at least as effective as normal T cells in helping B cells produce immunoglobulin molecules. This is evidence against the possibility that the helper activity is due to a small percentage of contaminating normal T cells in the Sézary cell population.

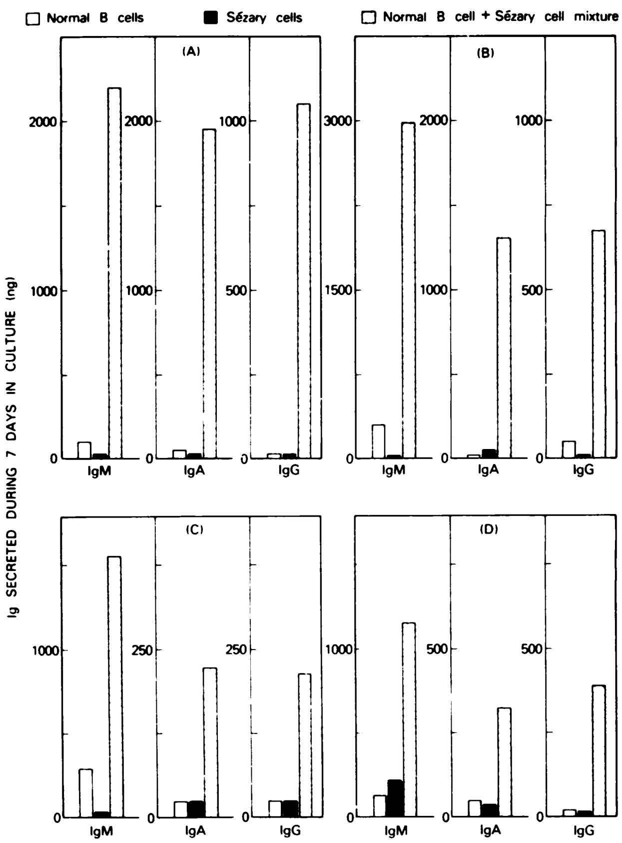


FIGURE 4 Immunoglobulin production by normal B cells alone, Sézary cells alone, and mixtures of normal B cells plus Sézary cells. Sézary cells were derived from the following patients: Experiment A, patient J. H.; Experiment B, patient R. B.; Experiment C, patient E. H.; and Experiment D, patient J. N. Note that Sézary cells from these patients demonstrate helper cell activity. In these experiments, 0.5 $\times 10^6$ B cells were co-cultured with $1.0 \times 10^6$ Sézary cells.

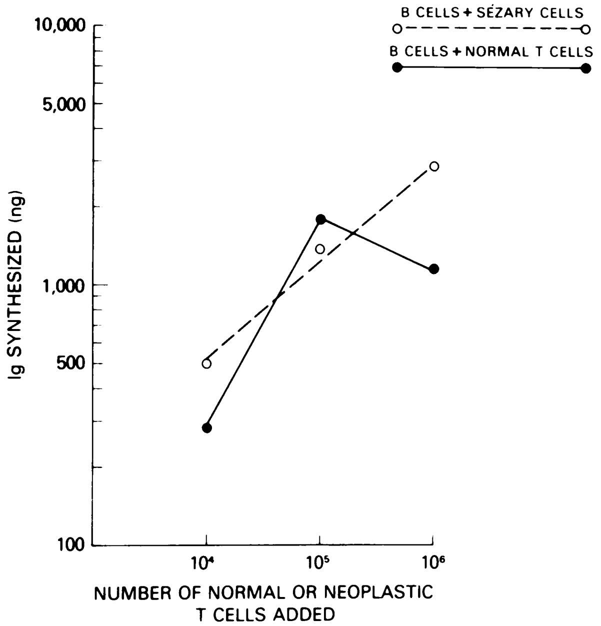


FIGURE 5 Immunoglobulin production by normal B cells in the presence of low numbers of normal T cells or Sézary cells. In these experiments $0.5 \times 10^6$ B cells were tested with varying dilutions of normal or neoplastic helper cell populations. Immunoglobulin values illustrated represent IgM. Sézary cells were from patient R. B.

We next examined the effect of adding progressively more Sézary cells ($4 \times 10^5$–$1.6 \times 10^6$ cells) to a constant number of B cells ($2 \times 10^5$ cells). The pattern of immunoglobulin synthesis by B cells with increasing numbers of neoplastic T cells contrasted with that observed when normal T cells were added. As noted above, when increasing numbers of normal T cells were added to purified normal B cells, relative suppression of pokeweed mitogen-induced immunoglobulin synthesis was observed at high T-cell to B-cell ratios. In contrast, as shown in Fig. 6, there was a progressive increase in the quantity of immunoglobulin synthesized by normal B cells with increases in the number of Sézary T cells added. The typical decline of immunoglobulin synthesis was not seen even at very high T-cell to B-cell ratios. This observation is in accord with the view that Sézary T cells represent pure helper cells, and not mixtures of suppressor and helper cells. This view is further supported by the observation that the addition of Sézary cells to mixtures of normal B cells and T cells could reverse the decline of immunoglobulin synthesis brought about by large concentrations of normal T cells. As noted in Fig. 7, the addition of a constant number of Sézary cells ($1 \times 10^6$ cells) to B-cell plus T-cell mixtures prevented the decline in immunoglobulin production associated with high normal T-cell to normal B-cell ratios.

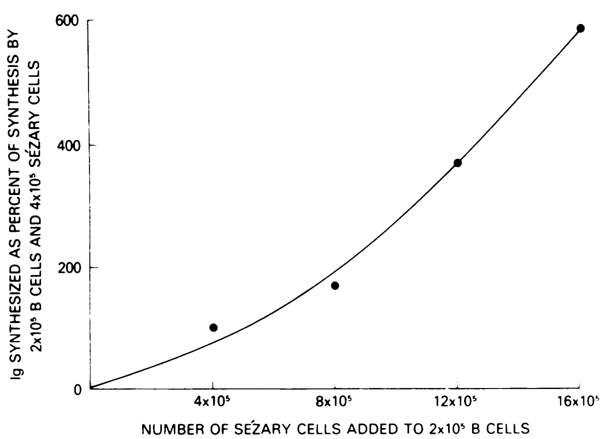


FIGURE 6 Immunoglobulin production by normal B cells as a function of the number of Sézary cells added to the culture. In the experiment illustrated, Sézary cells were from patient E. H. Immunoglobulin values illustrated represent IgA. Note, in contrast to the results using normal T cells (Fig. 3), immunoglobulin synthesis is not suppressed at high neoplastic T-cell to B-cell ratios.

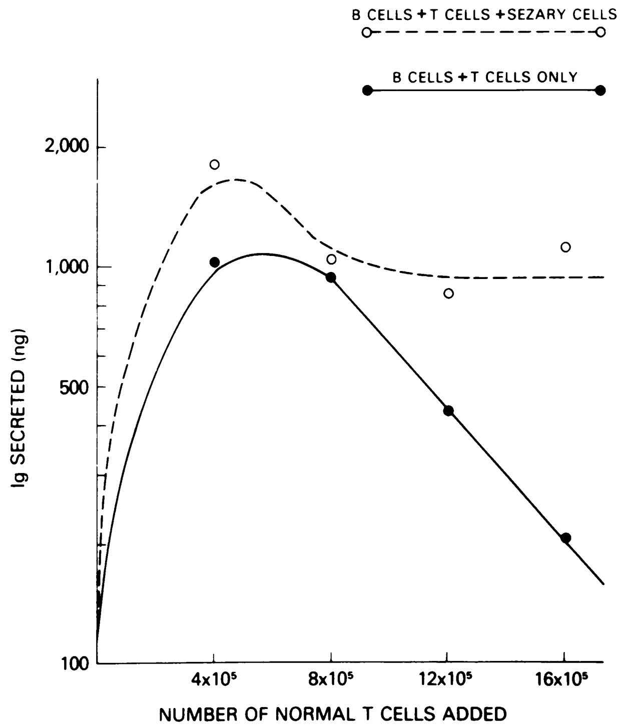


FIGURE 7 Restoration of immunoglobulin production at high normal T- and B-cell ratios by the addition of Sézary cells. In these experiments, varying numbers of normal T cells were added to $2 \times 10^5$ normal B cells. In addition, certain B cell—T cell mixtures received a constant number ($1.0 \times 10^6$) of Sézary cells derived from patient E. H. Immunoglobulin values illustrated represent IgA.

*Helper interaction of Sézary T cells with lymphocytes from patients with thymic deficiency states.* The majority of patients with immunoglobulin deficiencies do not have a lack of helper T cells as their fundamental defect, and the addition of normal T cells or Sézary cells does not augment immunoglobulin biosynthesis by lymphocytes of these patients in vitro. Two children with thymic deficiency states provided interesting exceptions to this generalization. The first patient was an 8-yr-old girl with Nezelof's syndrome (30) and an associated severe thymic deficiency as manifested by recurrent infections, absent delayed hypersensitivity, inability to reject skin allografts, and markedly decreased proliferative responses of peripheral blood lymphocytes when cultured in the presence of mitogens and specific antigens. This child also had reduced specific circulating antibody formation after repeated immunizations. As shown in Fig. 8 (top), the lymphocytes from this child were not capable of synthesizing IgG and IgA in the presence of pokeweed mitogen. However, when the lymphocytes from this thymic deficient child and lymphocytes from a Sézary syndrome patient (patient J. N.) were cocultured ($1 \times 10^6$ cells from each source), there was copious synthesis of IgG and IgA (more than 30,000 ng). The neoplastic Sézary T cells from this patient also promoted an increased immunoglobulin synthesis by the lymphocytes from a 7-yr-old child with ataxia telangiectasia (31) and selective IgA deficiency. The lymphocytes from this child produced only 120 ng of IgA when cultured alone in the presence of pokeweed mitogen. However, when 1 million Sézary cells were added to 1 million of the patient's cells, there was a 10-fold augmentation of IgA synthesis, thereby restoring the rate of IgA synthesis to normal (Fig. 8 bottom).

## DISCUSSION

Recently recognized membrane and functional differences between B and T cells have provided an elegant basis for classifying neoplastic lymphocytes. Such a classification has therapeutic and prognostic relevance, as in characterizing lymphomas and certain leukemias. In addition, the study of neoplastic lymphocytes, plasma cells, and their products has resulted in many new insights regarding the normal cells of the immune system and their effector proteins. This is especially true since these malignant cells appear to be unique

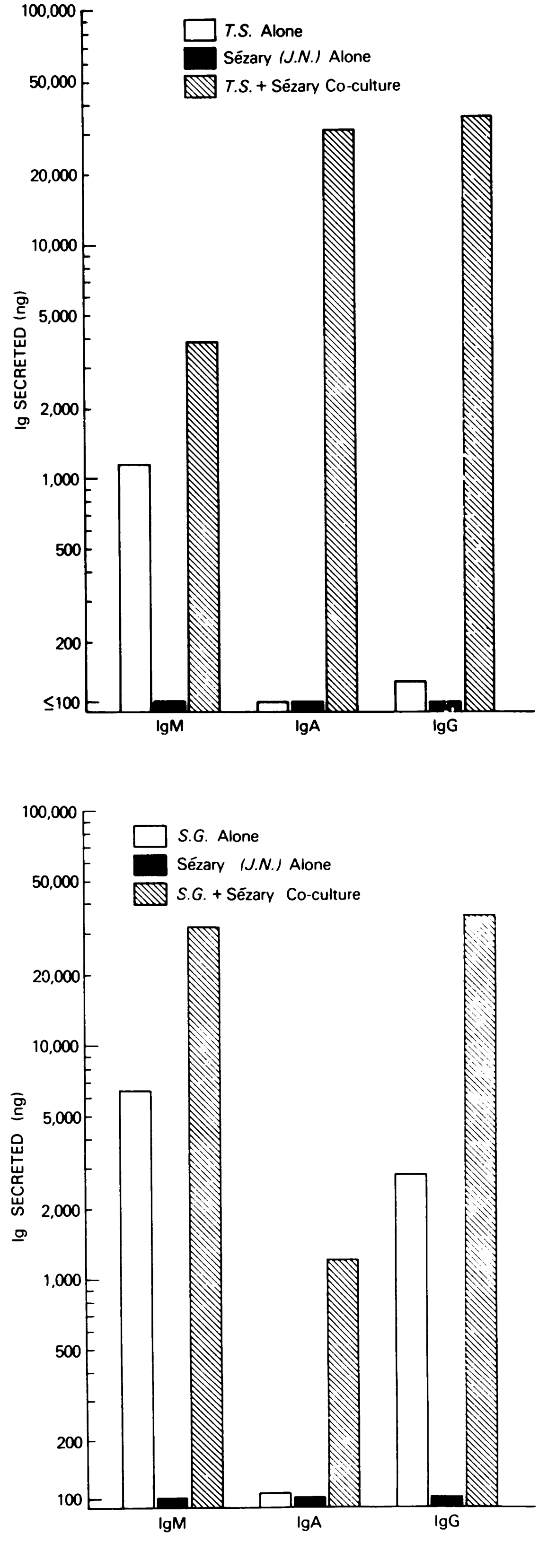


FIGURE 8 Helper interaction of Sézary cells with lymphocytes from patients with thymic deficiency states. T. S. was a child with Nezelof's syndrome. S. G. is a child with ataxia telangiectasia. The Sézary cells used in these experiments were from patient J. N.

clonal populations that cannot be easily obtained from normal individuals or from experimental animals. The study of the malignant T cells and their products from patients with the Sézary syndrome may prove to be as exciting in answering questions regarding the T-cell system as chronic lymphocytic leukemia cells, myeloma cells, and their products have proved to be in answering questions concerning the immunoglobulin synthesizing system.

One of the most critical questions concerning the T-cell regulation of immunoglobulin synthesis is whether a single population of T cells can mediate both helper and suppressor activity under appropriate conditions, or whether help and suppression are accomplished by two distinct populations of T cells, each genetically programmed to carry out only helper or suppressor functions. It has been suggested by several workers that suppressor T cells and helper T cells might be the same cells. Thus, suppression in any system has been viewed as either "too much help" (32) or a consequence of T-cell signals acting directly on B cells in the absence of macrophages (33). It has also been proposed that suppressor or helper effects depend upon the state of activation of the responding cells with stimulation taking place when the activity of the responders is low and suppression taking place when the activity of the responders is high (34). However, a considerable body of recent evidence indicates that there are two distinct groups of T cells, one programmed for helper function and the other programmed for suppressor function. The most clear-cut demonstration that suppressor T cells and helper T cells are distinct populations has been obtained by examining the genetically controlled Ly system of antigens in mice (35). These antigens represent markers expressed exclusively on the surface of cells undergoing thymus-dependent differentiation. Each of the Ly surface antigens is determined by a single genetic locus (Ly-1 on chromosome 19 and Ly-2 and Ly-3 closely linked on chromosome 6), having two alleles. With the use of specific antisera for the antigens of the Ly system, peripheral T cells can be subclassified on the basis of the differential expression of surface antigens belonging to the three Ly systems; Ly-1, Ly-2, and Ly-3. Three groups of T cells have been identified, one population bearing all three of the Ly determinants (Ly-123$^+$), one population bearing Ly-1 determinants alone, and a third population bearing Ly-23. It has been demonstrated that antisera to Ly-1 eliminated both antigen specific helper activity and concanavalin A-induced nonspecific helper T-cell activity, whereas antiserum to Ly-23 does not affect helper activity (36, 37). Thus, helper T cells or $T_H$ cells are Ly-1$^+$ cells. In contrast, antisera to Ly-1 does not affect either specific or nonspecific suppressor activity, whereas antisera to Ly-23 eliminated these functions. Thus, it appears from these studies that suppressor cells or $T_S$ cells bear Ly-23 determinants on their surface and are distinct from the helper T cells.

The results of the studies reported in this paper are in accord with the view that human helper cells are distinct from human suppressor cells. The Sézary cells of four of the five patients studied appeared to be a homogenous expansion of helper T cells ($T_H$ cells) comparable to the cells bearing the Ly-1 antigen in mice. The cells from these patients were capable of greatly augmenting the amount of immunoglobulin produced by purified B cells. The helper activity of the Sézary cells from patient J. N.[1] is of special interest since in previously published studies this patient's neoplastic T cells failed to proliferate in the presence of mitogens and specific antigens (1, 2). Furthermore, this patient's Sézary cells could not serve as stimulating or responding cells in mixed leukocyte reaction tests (1). Therefore, helper cell activity is not necessarily linked to other more commonly assayed T-cell functions. The previously reported studies in patient J. N., in conjunction with the control studies discussed above, indicate that the Sézary cell helper function reported here is not a simple example of the so-called allogeneic effect (19). The conclusion that the Sézary cells represent a homogeneous expansion of helper cells is supported by the results of adding graded increases of T cells to a constant number of B cells. When progressively more normal T cells were added to purified normal B cells, suppression of pokeweed mitogen-induced immunoglobulin production was observed at high T-cell to B-cell ratios. At least one explanation for this is that normal circulating lymphocyte populations contain both helper and suppressor T cells. In contrast, there was a progressive increase in the quantity of immunoglobulin synthesized by normal B cells when increasing numbers of Sézary cells were added, with no evidence of decline in immunoglobulin synthesis at very high neoplastic T-cell to B-cell ratios. Furthermore, the addition of Sézary cells to mixtures of normal B cells and normal T cells reversed the decline of immunoglobulin production caused by excessive numbers of normal T cells.

Three points are worth emphasizing. First, the relative suppression of immunoglobulin production at high normal T-cell to B-cell ratios is not due to simple crowding or exhaustion of essential tissue culture nutrients since the addition of even more cells to the system (i.e. Sézary cells) restored immunoglobulin production. Second, the helper function mediated by circulating lymphocytes from patients with the Sézary syndrome is not merely the result of residual normal

[1] Patient J. N. was previously labeled "Patient 1" (1).

helper T cells. Helper cell activity was seen with small numbers of Sézary cells. Furthermore, a small residual population of normal T cells would not be expected to totally overcome the suppressive effect of adding excessive numbers of T cells to B cells. Third, Sézary cells from certain patients appear to be exclusively programmed for helper interactions with B cells. Sézary cells from the patients studied thus far do not express suppressor activity in vitro. Continued research may show that Sézary cells from the majority of patients originate from lymphocytes which are the human counterparts of mouse $Ly\text{-}1^+$ T cells. However, it is likely that human lymphomas and leukemias originating from T cells corresponding to $Ly\text{-}23^+$ lymphocytes and expressing suppressor cell activity will be discovered eventually.

Sézary lymphocytes exerted profound helper-like activity when co-cultured with lymphocytes from a patient with severe thymic dysfunction and humoral antibody deficiency. Neoplastic T cells from the same patient also promoted a 10-fold increase in the in vitro synthesis of IgA by lymphocytes from a child with ataxia telangiectasia and selective IgA deficiency. These observations suggest that certain forms of humoral immunodeficiency may be due to a helper cell deficiency, and not only due to an intrinsic defect of the B cells alone or excessive numbers of suppressor cells. Indeed, it is exciting to consider that certain classes of neoplastic T cells, or soluble factors produced by such cells, could prove useful in the treatment of immunodeficiency states due to thymic dysfunction.

## ACKNOWLEDGMENT

We wish to thank Mrs. Karen Theoharis for excellent secretarial assistance.

## REFERENCES

1. Edelson, R. L., C. H. Kirkpatrick, E. M. Shevach, P. S. Schein, R. W. Smith, I. Green, and M. Lutzner. 1974. Preferential cutaneous infiltration by neoplastic thymus-derived lymphocytes. Morphologic and functional studies. *Ann. Intern. Med.* **80**: 685–692.
2. Lutzner, M., R. Edelson, P. Schein, I. Green, C. Kirkpatrick, and A. Ahmed. 1975. Cutaneous T-cell lymphomas: the Sézary syndrome, mycosis fungoides, and related disorders. *Ann. Intern. Med.* **83**: 534–552.
3. Braylan, R. C., E. S. Jaffe, and C. W. Berard. 1975. Malignant lymphomas: current classification and new observations. *Pathol. Annu.* **10**: 213–270.
4. Unanue, E. R., H. M. Grey, E. Rabellino, P. Campbell, and J. Schmidtke. 1971. Immunoglobulins on the surface of lymphocytes. II. The bone marrow as the main source of lymphocytes with detectable surface-bound immunoglobulin. *J. Exp. Med.* **133**: 1188–1198.
5. Pernis, B., M. Ferrarini, L. Forni, and L. Amante. 1971. Immunoglobulins on lymphocyte membranes. *In* Progress in Immunology. B. Amos, editor, Academic Press, Inc., New York. 95–106.
6. Nussenzweig, V., C. Bianco, P. Dukor, and A. Eden. 1971. Receptors for C3 on B lymphocytes: possible role in immune response. *In* Progress in Immunology. B. Amos, editor. Academic Press, Inc., New York 73–82.
7. Dickler, H. B., and H. G. Kunkel. 1972. Interaction of aggregated γ-globulin with B lymphocytes. *J. Exp. Med.* **136**: 191–196.
8. Grey, H. M., E. Rabellino, and B. Pirofsky. 1971. Immunoglobulins on the surface of lymphocytes. IV. Distribution in hypogammaglobulinemia, cellular immune deficiency, and chronic lymphatic leukemia. *J. Clin. Invest.* **50**: 2368–2375.
9. Aisenberg, A. C., and K. J. Bloch. 1972. Immunoglobulins on the surface of neoplastic lymphocytes. *N. Engl. J. Med.* **287**: 272–276.
10. Preud'homme, J. L., and M. Seligmann. 1972. Surface bound immunoglobulins as a cell marker in human lymphoproliferative diseases. *Blood.* **40**: 777–794.
11. Jondal, M., G. Holm, and H. Wigzell. 1972. Surface markers on human T and B lymphocytes. I. A large population of lymphocytes forming nonimmune rosettes with sheep red blood cells. *J. Exp. Med.* **136**: 207–215.
12. Fröland, S. S. 1972. Binding of sheep erythrocytes to human lymphocytes. A probable marker of T lymphocytes. *Scand. J. Immunol.* **1**: 269–280.
13. Smith, R. W., W. D. Terry, D. N. Buell, and K. W. Sell. 1973. An antigenic marker for human thymic lymphocytes. *J. Immunol.* **110**: 884–887.
14. Brouet, J-C., G. Flandrin, and M. Seligmann. 1973. Indications of the thymus-derived nature of the proliferating cells in six patients with Sézary's syndrome. *N. Engl. J. Med.* **289**: 341–344.
15. Lohrmann, H-P., L. Novikovs, and R. G. Graw, Jr. 1974. Cellular interactions in the proliferative response of human T and B lymphocytes to phytomitogens and allogeneic lymphocytes. *J. Exp. Med.* **139**: 1553–1567.
16. Lohrmann, H. P., and J. Whang-Peng. 1974. Human mixed leukocyte culture: identification of the proliferating lymphocyte subpopulation by sex chromosome markers. *J. Exp. Med.* **140**: 54–60.
17. Dennert, G. 1976. Thymus-derived killer cells: Specificity of function, and antigen recognition. *Transplant. Rev.* **29**: 59–88.
18. Yoshida, T., H. Sonozaki, and S. Cohen. 1973. The production of migration inhibitory factor by B and T cells of the guinea pig. *J. Exp. Med.* **138**: 784–797.
19. Katz, D. H., and B. Benacerraf. 1972. The regulatory influence of activated T cells on B cell responses to antigen. *Adv. Immunol.* **15**: 1–94.
20. Gershon, R. K. 1974. T cell control of antibody production. *Contemp. Top. Immunobiol.* **3**: 1–40.
21. Muchmore, A. V., R. M. Blaese, and L. Altman. 1976. Assessment of *in vitro* cell mediated immunity in chronic lymphocytic leukemia, a B-cell malignancy and the Sézary syndrome, a T-cell malignancy. *J. Immunol.* In press.
22. Yoshida, T., R. Edelson, S. Cohen, and I. Green. 1975. Migration inhibitory activity in serum and cell supernatants in patients with Sézary syndrome. *J. Immunol.* **114**: 915–918.
23. Wu, L. Y. F., A. R. Lawton, and M. D. Cooper. 1973. Differentiation capacity of cultured B lymphocytes from immunodeficient patients. *J. Clin. Invest.* **52**: 3180–3189.
24. Waldmann, T. A., M. Durm, S. Broder, M. Blackman, M. Blaese, and W. Strober. 1974. Role of suppressor T cells in pathogenesis of common variable hypogammaglobulinaemia. *Lancet.* **2**: 609–613.
25. Waldmann, T. A., S. H. Polmar, S. T. Balestra, M. C. Jost,

R. M. Bruce, and W. D. Terry. 1972. Immunoglobulin E in immunologic deficiency diseases. II Serum IgE concentration of patients with acquired hypogammaglobulinemia, thymoma and hypogammaglobulinemia, myotonic dystrophy, intestinal lymphangiectasia and Wiskott-Aldrich syndrome. *J. Immunol.* **109:** 304–310.
26. Chess, L., R. P. MacDermott, and S. F. Schlossman. 1974. Immunologic functions of isolated human lymphocyte subpopulations. I. Quantitative isolation of human T and B cells and response to mitogens. *J. Immunol.* **113:** 1113–1121.
27. Broom, B. C., E. G. De La Concha., A. D. B. Webster, G. J. Janossy, and G. L. Asherson. 1976. Intracellular immunoglobulin production *in vitro* by lymphocytes from patients with hypogammaglobulinaemia and their effect on normal lymphocytes. *Clin. Exp. Immunol.* **23:** 73–77.
28. Janossy, G., and M. Greaves. 1975. Functional analysis of murine and human B lymphocyte subsets. *Transplant. Rev.* **24:** 177–236.
29. Cooper, M. 1976. Discussion of T cell suppression of T and B cell mitogen-induced immunoglobulin production. *In* Mitogens in Immunobiology. J. J. Oppenheim and D. L. Rosenstreich, editors. Academic Press, Inc., New York. 528–529.
30. Nezelof, C. 1968. Thymic dysplasia with normal immunoglobulins and immunologic deficiency: pure alymphocytosis. *In* Immunologic Deficiency Diseases in Man. Birth Defects Original Article Series IV. D. Bergsma, editor. The National Foundation-March of Dimes, New York. 104–115.
31. McFarlin, D. E., W. Strober, and T. A. Waldmann. 1972. Ataxia-telangiectasia. *Medicine (Baltimore).* **51:** 281–314.
32. Coutinho, A., and G. Möller. 1975. Thymic-dependent B-cell induction and paralysis. *Adv. Immunol.* **21:** 113–236.
33. Feldmann, M. 1974. Antigen specific T cell factors and their role in the regulation of T-B interactions. *In* The Immune System: Genes, Receptors, Signals. E. E. Sercarz, A. R. Williamson, and C. F. Fox, editors. Academic Press, Inc., New York. 497–510.
34. Gershon, R. K. 1974. The "second law of thymodynamics." *In* The Immune System: Genes, Receptors, Signals. E. E. Sercarz, A. R. Williamson, and C. F. Fox, editors. Academic Press, Inc., New York. 471–484.
35. Cantor, H., and E. A. Boyse, 1975. T cell subclasses and regulation of the immune response. *In* Suppressor Cells in Immunity, International Symposium. S. K. Singhal and N. R. St. C. Sinclair, editors. University of Western Ontario Press, London, Canada. 34–38.
36. Jandinski, J., H. Cantor, T. Tadakuma, D. L. Peavy, and C. W. Pierce. 1976. Separation of helper T cells from suppressor T cells expressing different Ly components. I. Polyclonal activation: Suppressor and helper activities are inherent properties of distinct T-cell subclasses. *J. Exp. Med.* **143:** 1382–1390.
37. Cantor, H., F. W. Shen, and E. A. Boyse. 1976. Separation of helper T cells from suppressor T cells expressing different Ly components. II. Activation by antigen: after immunization, antigen-specific suppressor and helper activities are mediated by distinct T-cell subclasses. *J. Exp. Med.* **143:** 1391–1401.