Title

Successful treatment of autoimmunity in NZB/NZW F1 mice with monoclonal antibody to L3T4.

Journal

The Journal of experimental medicine, 161(2)

ISSN

0022-1007

Authors

Wofsy, D
Seaman, WE

Publication Date

1985-02-01

DOI

10.1084/jem.161.2.378

Peer reviewed

SUCCESSFUL TREATMENT OF AUTOIMMUNITY IN NZB/NZW F_1 MICE WITH MONOCLONAL ANTIBODY TO L3T4

BY DAVID WOFSY AND WILLIAM E. SEAMAN

From the Immunology/Arthritis Section, Veterans Administration Medical Center, and the Department of Medicine, University of California, San Francisco, California 94121

The advent of hybridoma technology has rekindled hopes of using antibodies as specific therapeutic agents (1–4). Several reports have focused attention on the potential use of monoclonal antibodies (mAb)[1] either as antitumor agents (4–15) or as immunosuppressive agents designed to facilitate organ transplantation (16–21). Early therapeutic trials in humans have demonstrated that certain mAb can be used to retard the progression of lymphoid malignancy (10, 11) or to reverse renal allograft rejection (17, 18). However, in most cases, the beneficial effects of treatment have been transient (4, 12–17) due in part to the development of host immunity to the administered mAb (4, 9, 16, 17).

Recently, (22) we treated three murine models for systemic lupus erythematosus (SLE) with biweekly injections of rat anti–T cell (anti-Thy-1.2) mAb in order to examine the role of T cells in the pathogenesis of murine lupus and to explore the possibility of using antilymphocyte mAb to treat autoimmunity. Treatment with anti-Thy-1.2 substantially reduced circulating T cells in all three strains (MRL/*lpr*, NZB/NZW F_1, BXSB), despite development of antibodies to rat Ig. Therapy was beneficial in MRL/*lpr* mice. It reduced autoantibody production, retarded renal disease, and markedly prolonged life. In contrast, treatment did not reduce autoimmunity in NZB/NZW F_1 (B/W) mice, and it caused fatal anaphylaxis in BXSB mice. These findings demonstrate that antilymphocyte mAb can serve as specific probes to examine the cells that contribute to autoimmunity. The results in MRL/*lpr* mice illustrate the potential therapeutic value of antilymphocyte mAb when a pathogenic cell subset can be identified. However, the results in BXSB mice emphasize equally the potential hazards of the host immune response to treatment with mAb.

The development of a new rat mAb (GK1.5) reactive with mouse helper T cells provides an opportunity for a more selective approach to the study of T cell regulation of autoimmunity in murine lupus. This antibody recognizes a glycoprotein antigen, designated L3T4, that is the mouse homologue for the human T cell antigen termed Leu-3 or T4 (23). Like Leu-3/T4 in humans, L3T4

This work was supported by the Veterans Administration, the State of California (contract 83-00062), and the Northern California Chapter of the Arthritis Foundation. Address reprint requests to: Dr. David Wofsy, Arthritis/Immunology Section (111R), Veterans Administration Medical Center, 4150 Clement Street, San Francisco, CA 94121.

[1] *Abbreviations used in this paper:* BGG, bovine gamma globulin; BSA, bovine serum albumin; BUN, blood urea nitrogen; ELISA; enzyme-linked immunosorbent assay; mAb, monoclonal antibody; MHA II, class II major histocompatibility antigens; SLE, systemic lupus erythematosus; TLI, total lymphoid irradiation.

participates in the T cell response to class II major histocompatibility antigens (MHA II) on antigen-presenting cells (24–28). Antibody to L3T4 inhibits most MHA II–dependent T cell responses in vitro (25–27). Therefore, administration of mAb against L3T4 might alter immune responses in vivo and thereby influence the course of autoimmune disease. To test this hypothesis, we treated B/W female mice with weekly injections of GK1.5 (anti-L3T4) beginning at age 4 mo. Treatment produced a sustained reduction in circulating T cells expressing L3T4. It also dramatically reduced autoantibody concentrations, retarded renal disease, and prolonged life. Mice treated with rat anti-L3T4 mAb developed little or no antibody to the administered rat Ig. In contrast, control mice treated with purified nonimmune rat IgG developed high titers of antibody to rat Ig. These findings establish that autoimmune disease in B/W mice is T cell–dependent. They further demonstrate that: (*a*) mAb directed against helper T cells can significantly improve autoimmune disease in B/W mice; and (*b*) the beneficial effects of treatment with anti-L3T4 may not be complicated by the development of a host immune response to therapy.

Materials and Methods

Mice. B/W mice were bred in our colony at the San Francisco Veterans Administration Medical Center from NZB females, purchased from The Jackson Laboratory, Bar Harbor, ME, and NZW males purchased from Simonsen Laboratory, Gilroy, CA

Treatment Regimen. 10 mice were treated with a rat IgG2b mAb against L3T4, designated GK1.5 (23). The hybridoma used to produce GK1.5 was generously provided by Dr. Frank W. Fitch, University of Chicago. Antibody to L3T4 was harvested as ascites from sublethally irradiated BALB/c mice, partially purified by ammonium sulfate precipitation, dialyzed against phosphate-buffered saline, and quantified by protein electrophoresis and measurement of optical density. Treatment consisted of an initial intravenous injection of 2 mg of anti-L3T4, followed by weekly intraperitoneal injections (2 mg per mouse). In preliminary studies, we determined that an intravenous injection of 2 mg of anti-L3T4 produces saturation binding of circulating and splenic target cells, and substantial, though incomplete, binding of $L3T4^+$ lymph node cells and thymocytes (unpublished data). Two control groups were also studied: one group (10 mice) received weekly 2-mg injections of chromatographically purified rat IgG (Cappel Laboratories, Cochranville, PA); the other group (11 mice) received saline.

Fluorescence Analysis of Lymphocyte Subpopulations. Peripheral blood was obtained from individual mice by retroorbital bleeding into heparinized pipettes. Mononuclear cells were separated by centrifugation over Lympholyte-M (Cedarlane Laboratories, Ontario, Canada), and quantified using a Coulter ZBI cell counter (Coulter Electronics, Inc., Hialeah, FL). Mononuclear cell subsets were identified by staining with fluorescein-conjugated mAb, as described previously (29). The antibodies used for analysis included: anti-Thy-1.2 (30-H12) to identify all T cells, anti-L3T4 (GK1.5) and anti-Lyt-2 (53-6) to identify the major T cell subsets, and anti-ThB (53-9) to identify B cells. Fluorescein-conjugated mouse anti–rat kappa chain mAb (MAR 18.5; B-D FACS Systems, Sunnyvale, CA) was used to detect anti–L3T4 on target cell surfaces. Except as noted, fluorescence analysis was always performed immediately before treatment, i.e., 1 wk after the previous injection.

Assessment of Renal Disease. Blood urea nitrogen (BUN) was determined on samples from individual mice using a CentrifiChem System 400 (Union Carbide, Danbury, CT). Proteinuria was measured colorimetrically by the use of Albustix (Miles Laboratories Inc., Elkhart, IN). This produces an approximation of proteinuria as follows: trace, 10 mg/dl; 1+, 30 mg/dl; 2+, 100 mg/dl; 3+, 300 mg/dl; 4+, 1,000 mg/dl. All measurements of proteinuria were performed by an observer who had no knowledge of the given treatment.

Measurement of Serum Antibodies to Double-stranded DNA. Anti-DNA antibody concentrations were assessed by a solid phase enzyme-linked immunosorbent assay (ELISA)

derived from the method of Fish and Ziff (30). Immulon II polyvinyl microtitration trays were coated sequentially with a 50 μg/ml solution of poly-L-lysine (Sigma Chemical Co., St. Louis, MO) in 0.1 M Tris-HCl buffer, pH 7.3 (75 μg/well), a 20 μg/ml solution of poly(dA-dT) (Sigma Chemical Co.) in Tris buffer (75 μl/well), and a 2% solution of bovine serum albumin (BSA) (Sigma Chemical Co.) in Tris buffer (250 μl/well). Serum samples that had been serially diluted in Tris buffer containing 1% BSA and 2% bovine gamma globulin (BGG) (Sigma Chemical Co.) were then incubated at 75 μl/well for 1.5 h at room temperature. The wells were then washed five times and incubated with peroxidase-conjugated goat anti-mouse Ig that had been extensively absorbed with rat Ig (0.3 μg/well; Cappel Laboratories). After incubation at room temperature for 1 h, the wells were washed five times and treated in the dark for 10 min with *o*-phenylenediamine (100 μl/well). The enzymatic reaction was stopped with 2 N H_2SO_4 (200 μl/well), and absorbance was measured at 490 nm. Serum from adult MRL/*lpr* and C57BL/6 mice served as positive and negative controls, respectively. The specificity of the assay was demonstrated using wells that had not been coated with poly(dA-dT).

Measurement of Serum Antibodies to Rat Ig. Antibodies to rat Ig were measured by an ELISA technique similar to the one described above. Briefly, Immulon II polyvinyl microtitration trays were coated with rat Ig mAb (0.5 μg 30-H12/well) followed by 2% BSA in 0.1 M Tris-HCl buffer. Serum samples were serially diluted in Tris buffer containing 1% BSA and 2% BGG, then incubated at 75 μl/well for 1.5 h at room temperature. The wells were then washed, incubated with peroxidase-conjugated goat anti-mouse Ig that had been absorbed with rat Ig, washed again, and then treated in the dark with *o*-phenylenediamine exactly as described above. Mouse anti-rat kappa chain mAb (MAR 18.5) and normal mouse serum served as positive and negative controls, respectively.

Measurement of Serum IgM and IgG Levels. Serum concentrations of mouse IgM and IgG were measured by ELISA. Immulon II polyvinyl microtitration trays were coated with a 10 μg/ml solution of either goat anti-mouse IgG or goat anti-mouse IgM (both, Cappel Laboratories), followed by 2% BSA in 0.1 M Tris-HCl buffer. Serum samples were serially diluted in Tris buffer containing 1% BSA and 2% BGG, then incubated at 75 μl/well for 1.5 h at room temperature. The wells were then washed, incubated with peroxidase-conjugated goat anti-mouse Ig that had been absorbed with rat Ig, washed again, and then treated in the dark with *o*-phenylenediamine, as described above. Purified mouse IgM and IgG (Miles Laboratories) were used to generate standard curves and document the specificity of the assay.

Results

Effect of Age on Circulating Lymphocyte Subpopulations in Untreated B/W Mice. In control B/W mice treated with saline, there was a spontaneous age-dependent reduction in circulating T lymphocytes (Fig. 1, *A* and *B*). The number of circulating B lymphocytes remained relatively constant (Fig. 1 *C*). Although T cell counts fell by ~50% between 4 and 8 mo of age, $L3T4^+$ cells and $Lyt\text{-}2^+$ cells were comparably affected and, therefore, the L3T4/Lyt-2 (helper/suppressor) ratio remained constant (Fig. 1 *D*). Treatment with weekly injections of nonimmune rat IgG did not affect the spontaneous progressive changes in circulating lymphocyte subpopulations in B/W mice.

Effect of Treatment With Anti-L3T4 mAb on Circulating Lymphocyte Subpopulations. Treatment of B/W mice with weekly injections of anti-L3T4 mAb produced a dramatic and sustained reduction in circulating $L3T4^+$ lymphocytes (Fig. 1 *A*). Circulating $L3T4^+$ cells were reduced by 30% 24 h after the first injection, by 90% 1 wk later, and by 95% thereafter. Fluorescence analysis of circulating lymphocytes using mouse anti-rat kappa chain mAb demonstrated persistence of administered antibody on the few remaining target cells 7 d after

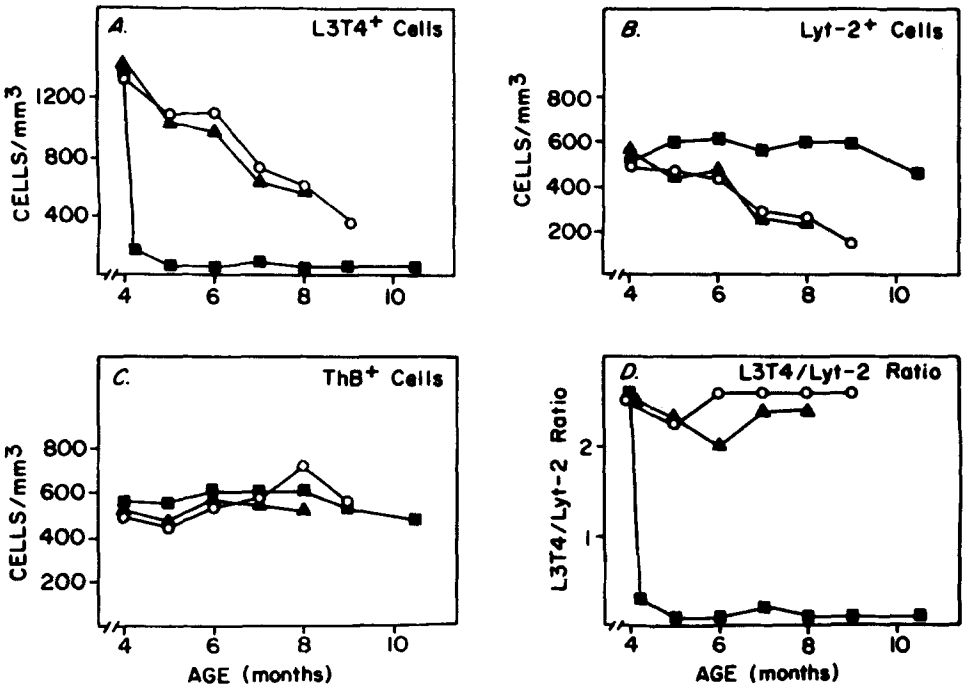

FIGURE 1. Circulating lymphocyte subpopulations in B/W mice treated with weekly injections of saline (○), nonimmune rat IgG (▲), or anti-L3T4 mAb (■) beginning at age 4 mo. Helper T cells, suppressor/cytotoxic T cells, and B cells were identified using fluorescein-conjugated anti-L3T4 mAb (*A*), anti-Lyt-2 (*B*), and anti-Thy B (*C*), respectively. The relative proportion of $L3T4^+$ and $Lyt\text{-}2^+$ T cells is shown in *D*.

injection. Staining with fluorescein-conjugated anti-Thy-1.2 mAb and anti-Lyt-2 established that, after 1 wk of therapy, >90% of circulating T cells in treated mice expressed Lyt-2, indicating that the reduction in $L3T4^+$ T cells reflected target cell depletion rather than antigen modulation. The effect of treatment with anti-L3T4 was specific. The number of circulating B cells and $Lyt\text{-}2^+$ T cells remained stable in treated mice (Fig. 1, *B* and *C*). The mean L3T4/Lyt-2 ratio in treated mice was 0.1:1, compared with 2.6:1, in untreated mice.

Host Immune Response to Treatment. Control mice treated with nonimmune rat IgG all developed high titers of antibody to rat Ig (Fig. 2). Host antibodies were detectable 7 d after the first injection of rat IgG and rapidly rose to a peak mean titer of 1:4,500. In contrast, only 3 of 10 mice treated with rat anti-L3T4 mAb developed antibodies to rat Ig. In these mice, antibodies to rat Ig first appeared after 2–3 mo of therapy and were present only in relatively low titer (<1:400). 7 of the 10 treated mice never developed detectable antibody to rat Ig. This is in striking contrast to our previous observation that B/W mice treated with rat anti-Thy-1.2 mAb of the same isotype as anti-L3T4 all develop high titers of anti-rat Ig (22).

Anti-L3T4 Reduces Autoimmunity. Treatment of B/W mice with anti-L3T4 mAb prevented development of high titers of antibody to double-stranded DNA (Fig. 3). Although small amounts of anti-DNA antibody appeared within the first

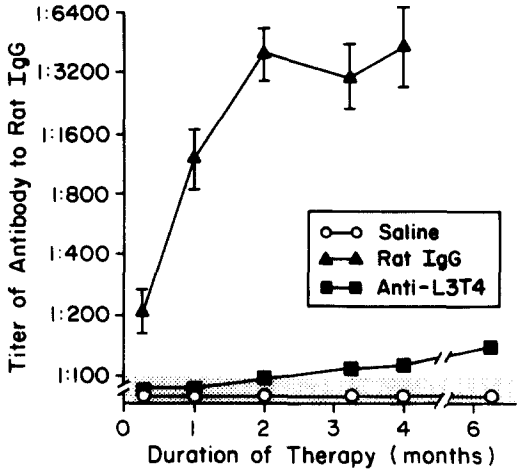

FIGURE 2. Geometric mean titer (± SEM) of antibodies to rat Ig in B/W mice treated with saline (○), nonimmune rat IgG (▲), or anti-L3T4 mAb (■). The shaded area indicates assay results using normal mouse sera as negative controls.

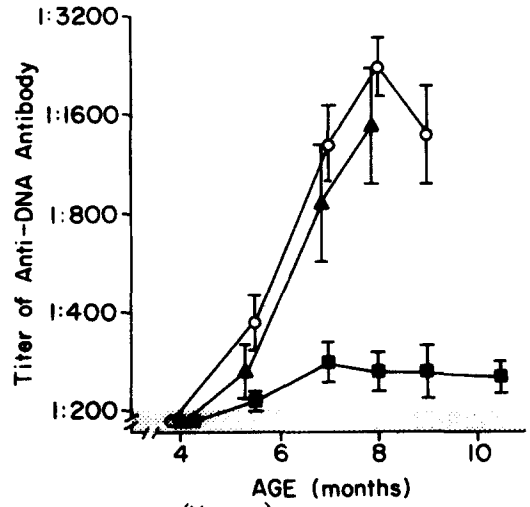

FIGURE 3. Geometric mean titer (± SEM) of antibody to double-stranded DNA in B/W mice treated with saline (○), nonimmune rat IgG (▲), or anti-L3T4 mAb (■). The shaded area indicates the titer of anti-DNA antibody (<1:200) in normal (C57BL/6) mice.

2 mo of therapy with anti-L3T4, the titers of anti-DNA antibody were markedly lower in treated mice than in control mice, and the titers remained low throughout the duration of therapy. At age 8 mo, the geometric mean titer of anti-DNA antibody was 1:2,200 ± 1.2 in mice treated with saline, 1:1,500 ± 1.5 in mice treated with nonimmune rat Ig, and 1:270 ± 1.1 in mice treated with anti-L3T4 ($P < 0.0005$ compared with either control group; Student's t test). The reduction in anti-DNA antibody did not reflect a generalized reduction in total Ig levels in treated mice (Table I). After 4 mo of treatment, there was a slight reduction in the mean IgG concentration in treated mice that was not statistically significant.

TABLE I
Effect of Treatment on Ig Concentration

Treatment	IgM*		IgG*	
	4-mo-old	8-mo-old	4-mo-old	8-mo-old
Saline	9.5 ± 0.2	9.2 ± 0.2	15.5 ± 0.2	15.7 ± 0.2
Rat IgG	9.6 ± 0.2	9.6 ± 0.2	15.7 ± 0.3	15.5 ± 0.6
Anti-L3T4	9.6 ± 0.2	10.3 ± 0.2‡	15.5 ± 0.3	15.2 ± 0.3

* Log_2 titer ($\bar{x}$ ± SEM) as measured by ELISA.
‡ $P < 0.05$ compared to pretreatment IgM levels and to IgM levels in both control groups.

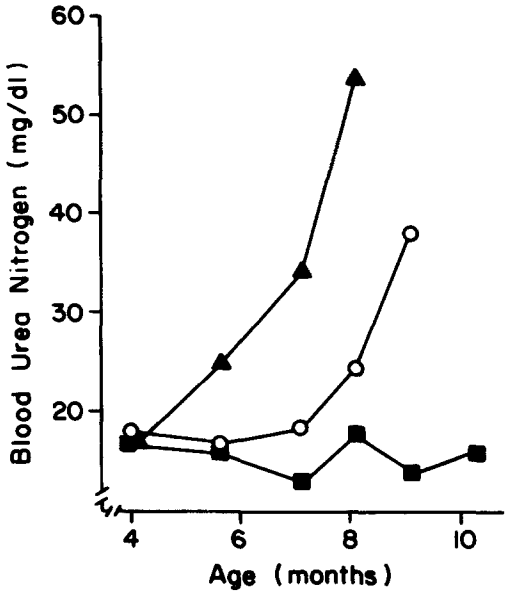

FIGURE 4. Mean BUN in B/W mice treated with saline (○), nonimmune rat IgG (▲) or anti-L3T4 mAb (■).

However, there was a significant increase in the mean IgM concentration in treated mice compared with pretreatment levels, and with IgM levels in control mice. Increased plasma IgM has been reported previously (31) in association with successful treatment of B/W mice.

Anti-L3T4 Reduces Renal Disease. The reduction in anti-DNA antibody concentration in mice treated with anti-L3T4 was associated with a significant reduction in renal disease. Renal function, as assessed by BUN, deteriorated steadily in both control groups, but was preserved in mice treated with anti-L3T4 (Fig. 4). Renal insufficiency occurred earliest in mice treated with nonimmune rat IgG, perhaps as a consequence of the host immune response to rat Ig. At age 7 mo, the mean BUN in mice treated with rat IgG was 34.2 ± 12.1 mg/dl, compared to 18.5 ± 2.7 mg/dl in mice treated with saline ($P < 0.05$; Mann-Whitney U test) and 13.1 ± 0.8 mg/dl in mice treated with anti-L3T4 ($P < 0.01$ compared with rat IgG treatment; $P < 0.05$ compared with saline treatment). By 9 mo, the mean BUN in mice treated with saline rose to 38.0 ± 11.6 mg/dl, compared with 14.3 ± 0.9 mg/dl in mice treated with anti-L3T4 (P

< 0.01). Severe proteinuria (≥3+) developed in 50–60% of mice in each control group, but in none of the mice treated with anti-L3T4 ($P < 0.05$; chi-square analysis) (Fig. 5). Moderate proteinuria (2+) developed in 16 of 21 control mice but in only 2 of 10 treated mice ($P < 0.05$). These two mice were among the three treated mice that developed low titers of antibody to rat Ig, suggesting that the immune response to rat Ig may have contributed to the development of proteinuria.

Anti-L3T4 Prolongs Survival. Treatment with anti-L3T4 mAb dramatically prolonged life (Fig. 6). Median survival was 9.3 mo in mice treated with saline and 7.6 mo in mice treated with rat IgG. In contrast, all of the mice treated with anti-L3T4 are still alive at 10 mo. The difference in 10-mo survival between

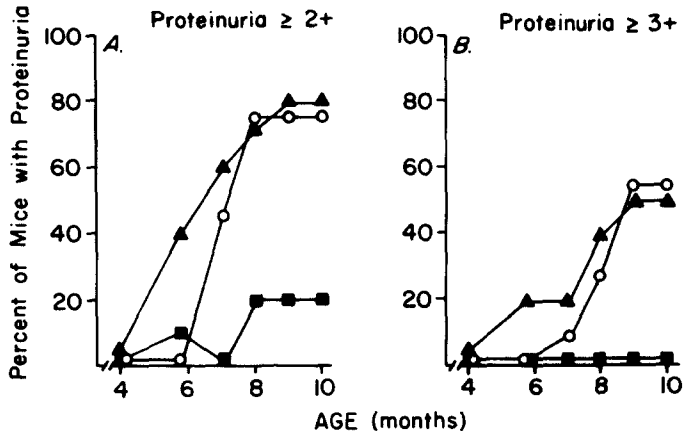

FIGURE 5. Frequency of significant proteinuria in B/W mice treated with saline (○), nonimmune rat IgG (▲), or anti-L3T4 mAb (■). (*A*) Percent of mice with proteinuria ≥2+ (100 mg/dl). (*B*) Percent of mice with proteinuria ≥3+ (300 mg/dl). To reflect accurately the development of renal disease in all mice, each point reflects the current level of proteinuria in surviving mice, as well as the last measurement of proteinuria in deceased mice.

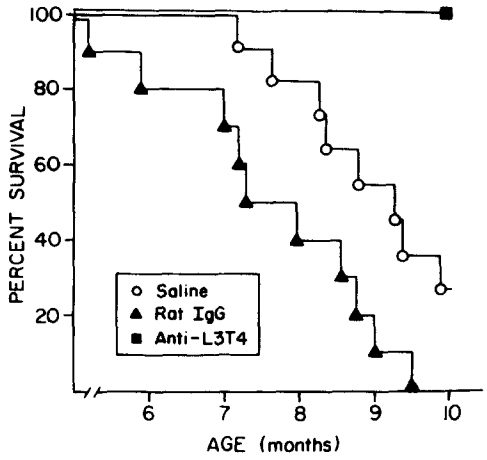

FIGURE 6. Survival in B/W mice treated with saline (○), nonimmune rat IgG (▲), or anti-L3T4 mAb (■).

mice treated with anti-L3T4 and control mice is strongly significant ($P < 0.001$ compared with the saline control group; $P < 0.00001$ compared with the rat IgG control group; chi-square analysis). The difference in mean survival between the two control groups is also statistically significant ($P < 0.05$; t test), i.e., treatment with nonimmune rat Ig accelerated mortality.

Discussion

To clarify the role of helper T cells in the pathogenesis of autoimmune disease in the B/W model for SLE, we treated female B/W mice with repeated injections of a rat mAb directed against the mouse helper T cell antigen, L3T4. Treatment depleted circulating target cells, reduced autoantibody production, retarded renal disease, and prolonged life. Moreover, treated mice produced little or no host antibodies to the administered rat mAb. These findings provide the first clear demonstration that autoimmune disease in B/W mice is T cell dependent. They further suggest that mAb directed against specific helper T cell antigens may be effective in the treatment of certain autoimmune diseases, and that the therapeutic benefit may be achieved without provoking an undesirable, and potentially hazardous, immune response to the mAb.

For two decades, the B/W strain has been studied (32, 33) as a model for SLE in humans. Early studies (34–37) focused attention on T cell abnormalities in B/W mice, emphasizing defects in suppressor T cell number and function. Recent studies, however, have cast doubt on the significance of these T cell abnormalities. For example, neonatal thymectomy has little effect on the course of autoimmunity in B/W mice (38), but elimination of a specific subset of B cells prevents autoimmune disease (39). Other studies, demonstrating B cell abnormalities, and indicating that B cell abnormalities may precede demonstrable T cell abnormalities in B/W mice, have suggested that autoimmunity in these mice may reflect a primary B cell defect, and that T cell defects may be secondary phenomena (40–43). Our results demonstrate conclusively that T cells, specifically helper T cells, play an important role in the pathogenesis of autoimmune disease in B/W mice. If primary defects occur in other cell types, particularly B lymphocytes, these cells nonetheless require helper T cells for the expression of disease.

It has been postulated that autoimmunity in B/W mice might reflect selective loss of suppressor T cells (36, 37), perhaps as a consequence of the action of natural thymocytotoxic antibodies (44, 45). Our findings in untreated B/W mice show that, although progressive T lymphocytopenia occurs, there is no selective effect on suppressor (Lyt-2^+) T cells. Rather, both major T cell subsets are comparably reduced, and the L3T4/Lyt-2 ratio is unchanged. There is, thus, no numerical imbalance between helper and suppressor/cytotoxic T cells, although functional differences cannot be excluded.

The L3T4 molecule is expressed on a distinct subpopulation of T cells previously referred to descriptively as helper T cells. More precisely, L3T4 identifies T cells that respond to MHA II on antigen-presenting cells (24–27). The functional importance of the L3T4 molecule is demonstrated by the observation that mAb against L3T4 blocks in vitro T cell responses to MHA II (25–27). The beneficial effects of anti-L3T4 in B/W mice may, therefore, be due not only to target cell depletion, but also to direct inhibition of T cell responses that

are dependent on the recognition of MHA II. The latter mechanism would imply that autoimmunity, like immunity to foreign antigens, requires a T cell response to MHA II. Our results are compatible with this possibility, but they do not prove it. We previously (22) treated B/W mice with mAb against Thy-1.2, an antigen expressed on all T cells. Although treatment reduced circulating $L3T4^+$ T cells by 75%, there was a comparable reduction in $Lyt\text{-}2^+$ T cells, and no improvement in autoimmunity. The failure of anti-Thy-1.2 to improve autoimmunity despite depletion of $L3T4^+$ cells suggests either that: (*a*) regulation of autoimmunity in B/W mice involves a balance between $L3T4^+$ cells and $Lyt\text{-}2^+$ cells that is not disturbed by treatment with anti-Thy-1.2; or (*b*) successful treatment with anti-L3T4 requires functional impairment of the L3T4 molecule.

An important observation in our present studies was the relative lack of host immunity to the anti-L3T4 antibody. Previous attempts to use mAb as therapeutic agents in humans and in mice have been complicated by the development of a host immune response to the mAb (4, 9, 16, 17, 22). In B/W mice treated with rat anti-Thy-1.2 mAb (subclass IgG2b), the development of antibody to rat Ig was associated with accelerated mortality, even though autoimmunity was not affected (22). In autoimmune BXSB mice, treatment with anti-Thy-1.2 caused fatal anaphylaxis (22). The current study reemphasizes the potential hazards of treatment with foreign Ig, because administration of purified nonimmune rat IgG significantly reduced survival. In this context, the weak host immune response to rat anti-L3T4 mAb (subclass IgG2b) takes on added significance. The ability to administer anti-L3T4 without generating high titers of host antibody to rat Ig may not only maximize the therapeutic effect of anti-L3T4, it may also minimize the risks associated with the host immune response to foreign protein.

The lack of host immunity to repeated injections of anti-L3T4 is the first demonstration that anti-L3T4 can interrupt the immune response in vivo. It remains to be determined if anti-L3T4 will block the in vivo response to foreign antigens other than itself, although it has been demonstrated (25) that anti-L3T4 can block in vitro responses to foreign antigens that are presented in association with MHA II. Immune recognition of MHA II can also be blocked both in vitro and in vivo by mAb against MHA II (46–49). One group of researchers (50–52) have treated murine models for several autoimmune diseases with mAb against MHA II. This treatment was effective in experimental autoimmune encephalitis (50) and experimentally induced myasthenia gravis (51). It also increased survival in B/W mice, even though it did not reduce autoantibody production (52). In mice, MHA II are expressed on macrophages, B cells, and activated T cells (53), but it is not known whether successful treatment with mAb against MHA II is associated with depletion of these cell types. In humans, MHA II are expressed not only on certain lymphoid cells, but also on blood vessel endothelial cells (54). This may account for the severe toxicity of mAb against MHA II in monkeys (55), which may complicate the use of anti-MHA II in humans. Unlike MHA II, L3T4 appears to be restricted in its expression to a distinct subpopulation of T lymphocytes and T lymphocyte precursors (24). Therefore, anti-L3T4 mAb may provide an alternative to the use of anti-MHA II mAb that would be more selective in its effect on the immune system. The human homologue for the

L3T4 antigen has been identified (23), and mAb against this antigen have already been used to prolong renal allograft survival in nonhuman primates (19).

Treatment of B/W mice with anti-L3T4 had profound effects on the immune system that raise questions about the immune competence of treated mice. The reduction in $L3T4^+$ T cells creates an imbalance between T cell subpopulations that resembles the distribution of T cell subsets in acquired immunodeficiency syndrome (AIDS) in people (56). The beneficial reduction in antibodies to double-stranded DNA and antibodies to rat Ig may reflect a more generalized impairment of humoral immunity. Our studies do not establish the significance of these alterations in cellular and humoral immunity, but they do provide some preliminary insight. Despite a 10-fold reduction in autoantibody concentration, treatment with anti-L3T4 did not significantly depress Ig levels. Moreover, treated mice did not develop infectious complications, even though they were housed without special precautions in our main animal colony. Studies are currently in progress in our laboratory to determine the precise effects of treatment with anti-L3T4 on immune competence in normal and autoimmune mice.

The immunologic abnormalities in B/W mice closely parallel the immunologic abnormalities underlying SLE in humans. T lymphocytopenia, impaired suppressor T cell function, reduced lymphokine production, and B cell hyperactivity all occur in humans with SLE as well as in B/W mice, but the significance of these abnormalities remains controversial (32, 57–60). There is no fixed abnormality in the relative proportion of circulating T cell subsets in either B/W mice or humans with SLE (61), but our results make it clear that this does not exclude the possibility that T cells regulate autoimmunity in SLE. The similarities between SLE in humans and SLE in B/W mice suggest that they may share common pathogenetic mechanisms. Our findings in B/W mice therefore support the hypothesis that autoimmunity in people with SLE is regulated by T cells. This hypothesis is consistent with recent studies indicating that total lymphoid irradiation (TLI) improves lupus nephritis in humans (62–64). Although the effect of TLI on lymphocyte subsets in SLE has not yet been determined, TLI causes prolonged depletion and impaired function of Leu-3/$T4^+$ cells in people with rheumatoid arthritis (65–67). These observations add weight to the possibility that treatment with mAb against Leu-3/T4 would favorably influence the course of autoimmune disease in people with SLE. However, we believe that such treatment would be premature until the effects of anti-L3T4 on normal immune function have been thoroughly investigated.

Summary

Autoimmune NZB/NZW mice were treated with weekly injections of monoclonal antibody (mAb) to L3T4, an antigen expressed on a distinct subpopulation of T cells that respond to class II major histocompatibility antigens. Treatment with anti-L3T4 depleted circulating target cells, reduced autoantibody production, retarded renal disease, and prolonged life relative to control mice treated either with saline or with purified nonimmune rat IgG. These findings establish that autoimmune disease in NZB/NZW mice is regulated by T cells. In contrast to mice treated with nonimmune rat IgG, mice treated with rat anti-L3T4 mAb

developed little or no antibody to rat Ig. Thus, the benefits of treatment with anti-L3T4 were achieved while minimizing the risks associated with a host immune response to therapy. This study raises the possibility that treatment with mAb against Leu-3/T4, the human homologue for L3T4, might be effective in the treatment of certain autoimmune diseases in people.

We thank Dr. Frank W. Fitch for the generous gift of hybridoma GK1.5. We also thank Ann Stembridge for her expert technical contribution and Denise Jang for her valuable assistance in preparation of the manuscript.

Received for publication 24 September 1984.

References

1. Ehrlich, P. 1906. Collected Studies on Immunity. Wiley & Sons, New York. 2:442–447.
2. Kohler, G., and C. Milstein. 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature (Lond.)* 256:495.
3. Milstein, C. 1980. Monoclonal antibodies. *Sci. Am.* 243:66.
4. Levy, R., and R. A. Miller. 1983. Tumor therapy with monoclonal antibodies. *Fed. Proc.* 42:2650.
5. Bernstein, I. D., M. R. Tam, and R. C. Nowinski. 1980. Mouse leukemia therapy with monoclonal antibodies against a thymus differentiation antigen. *Science (Wash. DC).* 207:68.
6. McGrath, M. S., E. Pillemer, and I. L. Weissman. 1980. Murine leukaemogenesis: monoclonal antibodies to T-cell determinants arrest T-lymphoma cell proliferation. *Nature (Lond.).* 285:259.
7. Weinstein, J. N., R. J. Parker, A. M. Keenan, S. K. Dower, H. C. Morse, III, and S. M. Sieber. 1982. Monoclonal antibodies in the lymphatics: toward the diagnosis and therapy of tumor metastases. *Science (Wash. DC).* 218:1334.
8. Krolick, K. A., D. Yuan, and E. S. Vitetta. 1981. Specific killing of a human breast carcinoma cell line by a monoclonal antibody coupled to the A-chain of ricin. *Cancer Immunol. Immunother.* 12:39.
9. Sears, H. F., D. Herlyn, Z. Steplewski, and H. Kaprowski. 1984. Effects of monoclonal antibody immunotherapy on patients with gastrointestinal adenocarcinoma. *J. Biol. Resp. Modif.* 3:138.
10. Miller, R. A., D. G. Maloney, R. Warnke, and R. Levy. 1982. Treatment of B-cell lymphoma with monoclonal anti-idiotype antibody. *N. Engl. J. Med.* 306:517.
11. Miller, R. A., and R. Levy. 1981. Response of cutaneous T cell lymphoma to therapy with hybridoma monoclonal antibody. *Lancet.* 2:226.
12. Nadler, L. M., P. Stashenko, R. Hardy, W. D. Kaplan, L. N. Button, D. W. Kufe, K. H. Autman, and S. F. Schlossman. 1980. Serotherapy of a patient with monoclonal antibody directed against a human lymphoma-associated antigen. *Cancer Res.* 40:3147.
13. Miller, R. A., D. G. Mahoney, J. McKillop, and R. Levy. 1981. In vivo effects of murine hybridoma monoclonal antibody in a patient with T-cell leukemia. *Blood.* 58:78.
14. Ritz, J., J. M. Pesando, S. E. Sallan, L. A. Clavell, J. Notis-McConarty, P. Rosenthal, and S. F. Schlossman. 1981. Serotherapy of acute lymphoblastic leukemia with monoclonal antibody. *Blood.* 58:141.
15. Dillman, R. D., D. L. Shawler, R. E. Sobol, H. A. Collins, J. C. Beauregard, S. B.

Wormsley, and I. Rayston. 1982. Murine monoclonal antibody therapy in two patients with chronic lymphocytic leukemia. *Blood.* 59:1036.

16. Colvin, R. B., A. B. Cosimi, R. C. Burton, J. T. Kurnick, C. Struzziero, G. Goldstein, and P. S. Russell. 1982. Anti-idiotype antibodies in patients treated with murine monoclonal antibody OKT3. *Fed. Proc.* 41:363.
17. Burton, R. C., A. B. Cosimi, R. B. Colvin, R. H. Rubin, F. L. Delmonico, G. Goldstein, and P. S. Russell. 1982. Monoclonal antibodies to human T cell subsets: use for immunological monitoring and immunosuppression in renal transplantation. *J. Clin. Immunol.* 2:1425.
18. Cosimi, A. B., R. B. Colvin, R. C. Burton, R. H. Rubin, G. Goldstein, P. C. Kung, W. P. Hansen, F. L. Delmonico, and P. S. Russell. 1981. Use of monoclonal antibodies to T cell subsets for immunologic monitoring and treatment in recipients of renal allografts. *N. Engl. J. Med.* 305:308.
19. Cosimi, A. B., R. C. Burton, P. C. Kung, R. Colvin, G. Goldstein, J. Lifter, W. Rhodes, and P. S. Russell. 1981. Evaluation in primate renal allograft recipients of monoclonal antibody to human T-cell subclasses. *Transplant. Proc.* 13:499.
20. Vallera, D. A., C. C. B. Soderling, G. J. Carlson, and J. H. Kersey. 1981. Bone marrow transplantation across major histocompatibility barriers in mice. *Transplantation (Baltimore).* 31:218.
21. Prentice, H. G., G. Janossy, D. Skeggs, H. A. Blacklock, K. F. Bradstock, G. Goldstein, and A. V. Hoffbrand. 1982. Use of anti-T-cell monoclonal antibody OKT3 to prevent acute graft-vs-host disease in allogeneic bone-marrow transplantation for acute leukaemia. *Lancet.* 1:700.
22. Wofsy, D., J. A. Ledbetter, P. L. Hendler, and W. E. Seaman. 1984. Treatment of murine lupus with monoclonal anti-T cell antibody. *J. Immunol.* In press.
23. Dialynas, D. P., Z. S. Quan, K. A. Wall, A. Pierres, J. Quintans, M. R. Loken, M. Pierres, and F. W. Fitch. 1983. Characterization of the murine T cell surface molecule, designated L3T4, identified by monoclonal antibody GK1.5: similarity of L3T4 to the human Leu-3/T4 molecule. *J. Immunol.* 131:2445.
24. Dialynas, D. P., D. B. Wilde, P. Marrack, A. Pierres, K. A. Wall, W. Havran, G. Otten, M. R. Loken, M. Pierres, J. Kappler, and F. W. Fitch. 1983. Characterization of the murine antigenic determinant, designated L3T4a, recognized by monoclonal antibody GK1.5: expression of L3T4a by functional T cell clones appears to correlate primarily with class II MHC antigen-reactivity. *Immunol. Rev.* 74:29.
25. Wilde, D. B., P. Marrack, J. Kappler, D. P. Dialynas, and F. W. Fitch. 1983. Evidence implicating L3T4 in class II MHC antigen reactivity: monoclonal antibody GK1.5 (anti-L3T4a) blocks class II MHC antigen-specific proliferation, release of lymphokines, and binding by cloned murine helper T lymphocyte lines. *J. Immunol.* 131:278.
26. Swain, S. L. 1983. T cell subsets and the recognition of MHC class. *Immunol. Rev.* 74:129.
27. Swain, G. L., D. P. Dialynas, F. W. Fitch, and M. English. 1984. Monoclonal antibody to L3T4 blocks the function of T cells specific for class II major histocompatibility complex antigens. *J. Immunol.* 132:1118.
28. Engleman, E. G., C. J. Benike, F. C. Grumet, and R. L. Evans. 1981. Activation of human T lymphocyte subsets: helper and suppressor/cytotoxic T cells recognize and respond to distinct histocompatibility antigens. *J. Immunol.* 127:2124.
29. Seaman, W. E., D. Wofsy, J. S. Greenspan, and J. A. Ledbetter. 1983. Treatment of autoimmune MRL/*lpr* mice with monoclonal antibody to Thy-1.2: a single injection has sustained effects on lymphoproliferation and renal disease. *J. Immunol.* 130:1713.
30. Fish, F., and M. Ziff. 1981. A sensitive solid phase microradioimmunoassay for anti-double stranded DNA antibodies. *Arthritis Rheum.* 24:534.

31. Seaman, W. E., M. A. Blackman, J. S. Greenspan, and N. Talal. 1980. Effect of ^{89}Sr on immunity and autoimmunity in NZB/NZW F_1 mice. *J. Immunol.* 124:812.
32. Steinberg, A. D., E. S. Raveche, C. A. Laskin, H. R. Smith, T. Santoro, M. L. Miller, and P. H. Plotz. 1984. Systemic lupus erythematosus: insights from animal models. *Ann. Intern. Med.* 100:714.
33. Theofilopoulos, A. N., and F. J. Dixon. 1981. Etiopathogenesis of murine SLE. *Immunol. Rev.* 55:179.
34. Steinberg, A. D. 1974. Pathogenesis of autoimmunity in New Zealand mice. V. Loss of thymic suppressor function. *Arthritis Rheum.* 17:11.
35. Barthold, D. R., S. Kysela, and A. D. Steinberg. 1974. Decline in suppressor T cell function with age in female NZB mice. *J. Immunol.* 112:9.
36. Krakauer, R. S., T. A. Waldmann, and W. Strober. 1976. Loss of suppressor T cells in adult NZB/NZW mice. *J. Exp. Med.* 144:662.
37. Cantor, H., L. McVay-Boudreau, J. Hugenberger, K. Naidorf, F. W. Shen, and R. K. Gershon. 1978. Immunoregulatory circuits among T-cell sets. II. Physiologic role of feedback inhibition in vivo: absence in NZB mice. *J. Exp. Med.* 147:1116.
38. Hang, L., A. N. Theofilopoulos, R. S. Balderas, S. J. Francis, and F. J. Dixon. 1984. The effect of thymectomy on lupus-prone mice. *J. Immunol.* 132:1809.
39. Steinberg, B. J., P. A. Smathers, K. Frederiksen, and A. D. Steinberg. 1982. Ability of the *xid* gene to prevent autoimmunity in (NZB × NZW)F_1 mice during the course of their natural history, after polyclonal stimulation, or following immunization with DNA. *J. Clin. Invest.* 70:587.
40. Moutsopoulos, H. M., M. Boehm-Truitt, S. S. Kassan, and T. M. Chused. 1977. Demonstration of activation of B lymphocytes in New Zealand black mice at birth by an immunoradiometric assay for murine IgM. *J. Immunol.* 119:1639.
41. Izui, S., P. J. McConahey, and F. J. Dixon. 1978. Increased spontaneous polyclonal activation of B lymphocytes in mice with spontaneous autoimmune disease. *J. Immunol.* 121:2213.
42. Primi, D., L. Hammarstrom, and C. I. E. Smith. 1978. Genetic control of lymphocyte suppression. I. Lack of suppression in aged NZB mice is due to a B cell defect. *J. Immunol.* 121:2241.
43. Prud'homme, G. J., R. S. Balderas, F. J. Dixon, and A. N. Theofilopoulos. 1983. B cell dependence on and response to accessory signals in murine lupus strains. *J. Exp. Med.* 157:1815.
44. Klassen, L. W., R. S. Krakauer, and A. D. Steinberg. 1977. Selective loss of suppressor cell function in New Zealand mice induced by NTA. *J. Immunol.* 119:830.
45. Shirai, T., K. Hayakawa, K. Okumura, and T. Tada. 1978. Differential effects of natural thymocytotoxic autoantibody on functional subsets of T cells. *J. Immunol.* 120:1924.
46. Perry, L. L., M. E. Dorf, B. A. Bach, B. Benacerraf, and M. I. Greene. 1980. Mechanisms of regulation of cell-mediated immunity. Anti-I-A alloantisera interfere with induction and expression of T-cell-mediated immunity to cell-bound antigen in vivo. *Clin. Immunol. Immunopathol.* 15:279.
47. Rosenbaum, J. T., N. E. Adelman, and H. O. McDevitt. 1981. In vivo effects of antibodies to immune response gene products. I. Haplotype-specific suppression of humoral immune responses with a monoclonal anti-I-A. *J. Exp. Med.* 154:1694.
48. Perry, L. L., and M. I. Greene. 1982. Conversion of immunity to suppression by in vivo administration of I-A subregion-specific antibodies. *J. Exp. Med.* 156:480.
49. Adelman, N. E., and H. O. McDevitt. 1982. Specific in vivo suppression of humoral responses with monoclonal anti-I-A antibodies. *Fed. Proc.* 41:592.
50. Steinman, L., J. T. Rosenbaum, S. Sririam, and H. O. McDevitt. 1981. In vivo effects

of antibodies to immune response gene products: preventive of experimental allergic encephalitis. *Proc. Natl. Acad. Sci. USA.* 78:7111.

51. Waldor, M. K., S. Sririam, H. O. McDevitt, and L. Steinman. 1983. In vivo therapy with monoclonal anti-I-A antibody suppresses immune responses to acteylocholine receptor. *Proc. Natl. Acad. Sci. USA.* 80:2713.
52. Adelman, N. E., D. L. Watling, and H. O. McDevitt. 1983. Treatment of (NZB × NZW)F_1 disease with anti-I-A monoclonal antibodies. *J. Exp. Med.* 158:1350.
53. Klein, J. 1979. The major histocompatibility complex of the mouse. *Science* (*Wash. DC*). 203:516.
54. Baldwin, W. M., F. H. Class, L. A. van Es, and J. J. van Rood. 1982. Distribution of endothelial-monocyte and HLA antigens on renal vascular endothelium. *Transplant. Proc.* 13:103.
55. Chatterjee, S., D. Bernoco, and R. Billing. 1982. Treatment with anti-Ia and antiblast/monocyte monoclonal antibodies can prolong skin allograft survival in nonhuman primates. *Hybridoma.* 5:369.
56. Gottlieb, M. S., J. E. Groopman, W. M. Weinstein, J. L. Fahey, and R. Detels. 1983. The acquired immunodeficiency syndrome. *Ann. Intern. Med.* 99:208.
57. Fauci, A. S., A. D. Steinberg, B. F. Haynes, and G. Whalen. 1978. Immunoregulatory aberrations in systemic lupus erythematosus. *J. Immunol.* 121:1473.
58. Miller, K. B., and R. S. Schwartz. 1979. Familial abnormalities of suppressor-cell function in systemic lupus erythematosus. *N. Engl. J. Med.* 301:803.
59. Blaese, R. M., J. Grayson, and A. D. Steinberg. 1980. Elevated immunoglobulin-secreting cells in the blood of patients with active systemic lupus erythematosus: correlation of laboratory and clinical assessment of disease activity. *Am. J. Med.* 69:345.
60. Linker-Israeli, M., A. C. Bakke, R. C. Kitridou, S. Gendler, S. Gillis, and D. A. Horowitz. 1983. Defective production of interleukin 1 and interleukin 2 in patients with systemic lupus erythematosus. *J. Immunol.* 130:2651.
61. Smolen, J. S., T. M. Chused, W. M. Leiserson, J. P. Reeves, D. Alling, and A. D. Steinberg. 1982. Heterogeneity of immunoregulatory T-cell subsets in systemic lupus erythematosus. Correlation with clinical features. *Am. J. Med.* 72:783.
62. Field, E., S. Strober, O. Shemesh, B. Kotzin, R. Hoppe, S. Friedman, E. Engleman, H. Kaplan, and B. Myers. 1984. Treatment of membranoproliferative lupus nephritis with total lymphoid irradiation (TLI). *Arhritis Rheum.* 27:S20.
63. Tanay, A. S., and S. Strober. 1984. Opposite effects of total lymphoid irradiation (TLI) on T cell dependent and T cell independent antibody responses in mice and humans. *Arthritis Rheum.* 27:S29.
64. Tanay, A. S., E. H. Field, and S. Strober. 1984. Opposite effects of total lymphoid irradiation (TLI) on serum levels of autoantibodies in patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) nephritis. *Arthritis Rheum.* 27:S61.
65. Kotzin, B. L., G. S. Kansas, E. G. Engleman, R. T. Hoppe, H. S. Kaplan, and S. Strober. 1983. Changes in T-cell subsets in patients with rheumatoid arthritis treated with total lymphoid irradiation. *Clin. Immunol. Immunopathol.* 27:250.
66. Brahn, E., S. Melfgott, J. A. Belli, R. J. Anderson, E. L. Reinherz, S. F. Schlossman, K. F. Austen, and D. E. Trentham. 1984. Total lymphoid irradiation therapy in refractory rheumatoid arthritis. Fifteen- to forty-month follow-up. *Arthritis Rheum.* 27:481.
67. Field, H., E. Engleman, and S. Strober. 1984. Reduced in vitro immune responses of purified human Leu 3 cells after total lymphoid irradiation. *Arthritis Rheum.* 27:S58.