trees extracting CO_2 from the atmosphere. This model implies that, without concurrent deforestation in this century, the buildup of atmospheric CO_2 in the atmosphere would have been very minor at best. The draining of the cellulose reservoir has no effect on earlier interpretations of the Suess effect in which the ^{12}C and ^{13}C from fossil fuel dilute the ^{14}C in the atmosphere; on the average, the ^{14}C in the carbon of the cellulose reservoir is equal, within experimental error, to that in the atmosphere.

One may obtain refined estimates of the amounts of CO_2 released to the atmosphere by the burning of wood in the past century by analyzing certain polycyclic aromatic compounds contained in glacier ice or sediments. Polycyclic aromatic hydrocarbons formed during nonindustrial burning of wood are distinct from those formed by either living organisms or the burning of fossil fuel (*13*); hence, they may provide a useful molecular tracer.

Prudent concern for the future would suggest the need for (i) further reexamination of the quantitative aspects of net deforestation and wood burning as they affect the amount of CO_2 in the atmosphere and atmospheric inputs and outputs (*14*); (ii) reexamination of the possible effects of the buildup of atmospheric CO_2 (*15*); and (iii) evaluation of the potential benefits of reforestation, not only for esthetic purposes but also as a way of stabilizing the wood biomass and atmosphere, providing a steady-state source of material and fuel.

J. A. S. ADAMS*
M. S. M. MANTOVANI
Instituto Astronômico e Geofísico, Universidade de São Paulo, Caixa Postal 30.627, São Paulo, Brazil
L. L. LUNDELL†
Department of Geology, Rice University, Houston, Texas 77001

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* On leave from Rice University, Houston, Tex. 77001 during 1976.
† Present address: Atlantic-Richfield Company, P.O. Box 2819, Dallas, Tex. 75221.

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Prevention of Autoimmunity in Experimental Lupus Erythematosus by Soluble Immune Response Suppressor

Abstract. *Young NZB/W mice, treated with injections of soluble immune response suppressor (SIRS) (supernatant from mouse spleen cells exposed to concanavalin A), showed decreased immunoglobulin levels, less antibody to cell nuclei, less proteinuria, and less renal pathology as compared with NZB/W mice receiving a control preparation. Thus, SIRS administration beginning at an early age appears to be an effective therapy of the autoimmune disease in NZB/W mice.*

NZB/W mice have been used as an animal model of human systemic lupus erythematosus (SLE) because animals of this strain exhibit pathologic and immunologic changes characteristic of this disease. Thus NZB/W mice develop antibodies to cell nuclei (antinuclear antibodies) (*1*), occasional Coomb's positive hemolytic anemia (*2*), immune complex glomerulonephritis (*1*), proteinuria (*1*), and vasculitis (*1*). As a result of the similarity between NZB/W mice and patients with SLE, an understanding of the mechanism responsible for the autoimmunity in NZB/W mice as well as the development of a rational therapeutic scheme for the autoimmunity in NZB/W mice is likely to have important consequences for the understanding and treatment of human SLE.

Evidence has accumulated implicating loss of suppressor T cell activity as an important underlying factor in the pathogenesis of the autoimmune disease in NZB/W mice (*2, 3*). This evidence includes data obtained in our laboratory (*4*) showing that suppressor T cells can be induced by concanavalin A in cultures of spleen cells obtained from normal animals and from young NZB/W animals, but not from concanavalin A–stimulated spleen cells obtained from adult NZB/W animals. Moreover, normal cells and cells from young NZB/W animals could be induced by concanavalin A to form a soluble immune response suppressor, whereas adult NZB/W spleen cells produced much less of this material. Finally, although spleen cells from young and adult NZB/W mice differ in their suppressor cell potential, cells from both sources could respond equally to suppressor signals in that concanavalin A–exposed normal cells or supernatants derived from such cells caused equivalent suppression of pokeweed mitogen–stimulated immunoglobulin synthesis by young and adult NBZ/W cells. These studies, in conjunction with others, point to the fact that NZB/W mice lose suppressor T cell activity as they age, yet retain the capacity to respond to negative regulatory signals. These observations suggest the possibility that such mice could be treated with the suppressor T cell product, soluble immune response suppressor (SIRS). Accordingly, we set up a clinical trial to determine whether the in vivo administration of SIRS could prevent the development of the manifestations of autoimmunity that are observed in untreated NZB/W mice.

A group (60) of 4-week-old female NZB/W F_1 mice (NZB female × NZW male animals) were segregated into four subgroups. Group A was treated with the supernatant of concanavalin A–activated spleen cells from adult BALB/c animals (BALB/c SIRS); group B was treated with the supernatant of concanavalin A–activated spleen cells of young (4-week-old) NZB/W spleen cells (NZB/W SIRS). Group C was a control group to validate the adequacy of the removal of concanavalin A from the supernatants. The animals of this group were treated with supernatants of BALB/c spleen cells exposed to concanavalin A for only a few minutes. Finally, the animals in group D were untreated. Animals treated with supernatants of concanavalin A–activated spleen cells (SIRS) were injected intraperitoneally three times weekly with 0.5 ml of absorbed supernatants of the cultured spleen cells prepared according to a method adapted from that of Rich and Pierce (*5*). Every 6 weeks three animals (so designated from the beginning of the study) in each group were killed and studied. The effects of administration of supernatants of spleen cells incubated with concanavalin A (SIRS) on the se-

rum immunoglobulin levels and on the development of circulating antinuclear antibody, of Coomb's positive hemolytic anemia, of renal pathology, and of proteinuria were determined when the animals were killed. A second group of 60 mice were segregated (in the manner indicated above for the first group), kept in separate cages, and observed for the effects of treatment on survival.

Treatment of NZB/W mice with SIRS derived from BALB/c mice or from young NZB/W animals led to hypogammaglobulinemia as well as a decrease in autoimmune antibody. With regard to the hypogammaglobulinemia, serum immunoglobulin (Ig) was measured by radial immunodiffusion (*6*), and animals from the two treatment groups showed a reduction of approximately 75 percent in the geometric means of the serum concentrations of IgG_{2a} and IgA and a 50 percent reduction of the serum IgM as compared either to animals receiving a control preparation in which spleen cells had been incubated with concanavalin A for only a few minutes or to animals that were untreated (Fig. 1). In spite of this induced hypogammaglobulinemia, none of the treated animals developed clinically apparent infections.

With regard to the effect of treatment on the development of autoantibodies, it was observed that none of the animals receiving BALB/c or young NZB/W SIRS developed antibodies to red blood cells as detected by the direct Coomb's test, whereas 47 percent of the untreated animals and 42 percent of the animals treated with control supernatant developed such antibodies to red cells after age 16 weeks (12 weeks after initiation of SIRS therapy). Furthermore, the development of antibodies to cell nuclei as assessed by the method of Cleymaet and Nakamura (*7*) was also decreased. As noted in Table 1, at each time point studied, antibodies to nuclei were absent or present at an exceedingly low level (+/−) in the animals treated with SIRS, but were positive in the untreated animals or those treated with control supernatants. Thus, SIRS treatment not only reduced immunoglobulin levels in general but also suppressed production of autoantibody.

A more critical question than the effect of SIRS treatment on the development of antibodies to cell nuclei or to red cells is whether SIRS administration can prevent or retard the development of autoimmune renal disease resulting from deposition of the immune complex. Accordingly, at 6-week intervals, designated mice in all four groups were killed; the kidneys were sectioned, coded, and examined for evidence of glomerulonephritis. The sections were graded 0 to 4+ on the basis of severity of lesions; hypercellularity, mesangial proliferation, necrosis, wire loop formation, protein deposition, and adhesions were considered evidence of glomerular disease. Animals treated with SIRS showed less renal pathology than those that were untreated or treated with control supernatants (Fig. 2 and Table 2).

For a more quantitative measure of renal disease, 24-hour urine samples were collected and pooled from three mice at age 34 weeks of each study group. The protein content of these pooled samples was then determined by the method of Lowry *et al.* (*8*). The SIRS–treated BALB/c mice excreted 22 ± 24 mg of protein per 100 ml of urine, and the young NZB/W mice of the SIRS–treated group excreted 48 ± 13 mg/100 ml, whereas the supernatant–treated control animals excreted 105 ± 28 mg/100 ml, and the untreated group excreted 112 ± 31 mg/100 ml. Thus, SIRS treatment can in large measure prevent the development of renal glomerular abnormalities resulting in proteinuria in NZB/W mice.

Finally, the effect of SIRS treatment on animal survival was evaluated. It was observed that, whereas 14 of 15 animals in each of the treated groups are still surviving (at 40 weeks of age), 13 of 15 of the untreated and 14 of 15 of the animals treated with the control supernatant died in their 20th to 30th week, the usual time of death for untreated NZB/W mice.

A considerable number of therapeutic approaches have been taken in the attempt to treat the autoimmune disease observed in NZB/W mice. Taking the chemotherapeutic approach, other investigators treated NZB/W mice with cyclophosphamide, azathioprine, or corticosteroids. However, these therapies have not been entirely satisfactory in that they have been associated with a number of complications. In addition, although the net effect on the immune response and host inflammation can be

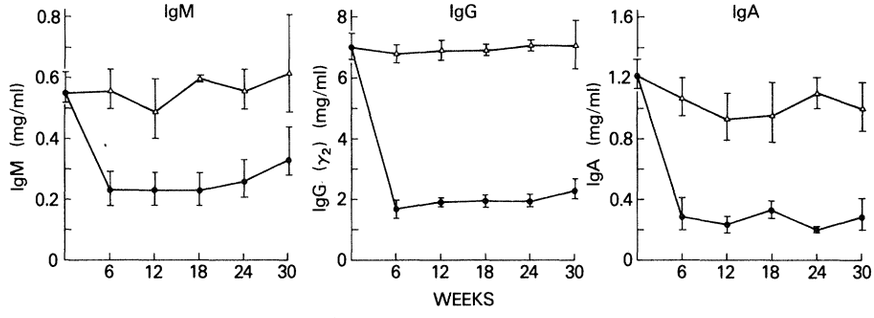

Fig. 1. Comparison of relative geometric mean concentrations of mouse immunoglobulins, IgG(γ_2), IgA, and IgM between those treated with a control SIRS preparation (△), and those treated with BALB/c SIRS (●), during the length of the study. Time (weeks) is from the beginning of treatment (age 4 weeks). Control SIRS consists of supernatant obtained from BALB/c spleen cells exposed to concanavalin A for several minutes. The immunoglobulin levels of the group of animals treated with young (4-week-old) NZB/W SIRS were similar to those of animals treated wtih BALB/c SIRS.

Table 1. Effects of SIRS treatment on presence of anti-nuclear antibody in individual NZB/W mice at various times after initiation of SIRS treatment at 4 weeks of age. The serums were assayed to a 1 to 10 dilution.

SIRS preparation	Effects on mice of age (weeks):				
	10	16	22	28	34
BALB/c spleen	−	−	−	−	−
	−	−	−	−	−
	−	−	−	−	
Young NZB/W (4-week) spleen	+/−	+/−	+/−	−	−
	+/−	+/−	−	+/−	−
	+/−	+/−	+/−	+/−	
Control SIRS*	+	+	+	+	+
	+	+	+	+	+
	+	+	+	+	
Untreated	+	+	+	+	+
	+	+	+	+	+
	+	+	+	+	

*Control SIRS consists of supernatant obtained from BALB/c spleen cells exposed to concanavalin A for several minutes.

beneficial, these therapies further depress the activity of suppressor T cells (*9*) and thus might aggravate one of the underlying causes of the autoimmunity.

Various attempts were made by others, using still another therapeutic approach, toward augmenting the capacity of older NZB/W to manifest suppressor T cell activity. One such attempt involves the use of thymosin and is related to the observation of Dauphinee, Talal, Goldstein, and White (*10*) that both thymosin levels in NZB/W mice and the proliferative response of NZB/W thymocytes in allogeneic recipients decrease as the animals age. They found that thymosin does in fact correct the proliferative response of older NZB/W thymocytes; nevertheless, thymosin administration (with the use of various schedules starting at different ages) does not result in a dramatic delay in the onset of autoimmunity (*11*). In another attempt to augment suppressor T cell activity, Gershwin and Steinberg (*12*) treated NZB/W mice in vivo with concanavalin A. They observed that such treatment given to young mice does result in a more prolonged tolerance to foreign protein antigens than is observed in untreated NZB/W mice, but nevertheless the animals lost tolerance at a time when normal animals maintain tolerance. This relative failure of concanavalin A treatment is in accord with our previous study (*4*), where we showed that there is a progressive decline in the number of cells in the spleens of NZB/W mice that can be activated to become suppressor cells by concanavalin A treatment. Finally, NZB/W autoimmunity has been treated by the administration of thymocytes or splenocytes from young NZB/W mice (*13*). Such treatment inhibited the development of Coomb's positive hemolytic anemia, but was only marginally successful in preventing other autoimmune phenomena. In addition, initiation of young thymocyte treatment at 10 weeks of age when the autoimmunity was becoming established rather than at 4 weeks of age was ineffective. This may be due to the relatively early development in NZB/W animals of cytotoxic natural antibodies to thymocytes that destroy the transferred T cells (*14*). In any case, administration of T cells to unrelated individuals is not likely to be a successful long-term treatment in humans because of histoincompatability.

The demonstration in our previous studies (*4*) that there was progressive decline in the production of suppressor T cells and of SIRS by concanavalin A–stimulated spleen cells of adult NZB/W mice, but retention of the response to these negative signals produced by normal mouse T cells, raised the possibility of treating NZB/W mice with SIRS and thus replacing the diminished physiological regulator. Our data show that treatment with SIRS given parenterally can lead to a significant decrease in immunoglobulin levels and can prevent or delay several aspects of autoimmunity in the NZB/W mouse. Furthermore, this effect on autoimmunity translates into a reduction in the severity of the renal lesions as well as prolonged survival and is achieved without evidence of serious toxicity. These effects of SIRS treatment on the autoimmune disease of NZB/W mice encourage the belief that treatment of patients with systemic lupus erythematosus with human SIRS may become a valuable therapeutic approach.

Table 2. Effects of treatment with SIRS on renal pathology (graded 0 to 4+) encountered in individual NZB/W mice at different time points after initiation of SIRS treatment at 4 weeks of age.

Preparation	Renal pathology in mice of age (weeks)				
	10	16	22	28	34
BALB/c SIRS	+	+	0	++	+
	+	0	0	0	0
	0	+	+	0	0
Young (4-week) NZB/W SIRS	+	+	+	+	0
	+	+	0	+	+
	+	+	+	+	+
Control preparation*	++	++	++	+++	++++
	++	++	++	+++	+++
	+	++	+++	+++	+++
Untreated	+	++	++	++++	++++
	++	+	+++	+++	+++
	+	++	++	+++	++++

*Control preparation consists of supernatant fluid obtained from BALB/c spleen cells exposed to concanavalin A for several minutes.

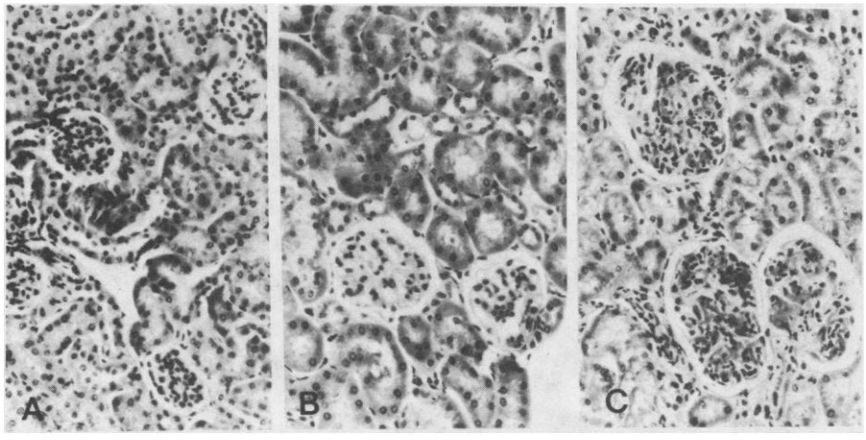

Fig. 2. Representative kidney sections of mice at 30 weeks of treatment (age 34 weeks). (A) Mice treated with BALB/c SIRS. (B) Mice treated with young (4-week-old) NZB/W SIRS. (C) Mice treated with a control SIRS preparation (supernatant obtained from BALB/c spleen cells exposed to concanavalin A for several minutes).

R. S. KRAKAUER,* W. STROBER
D. L. RIPPEON, T. A. WALDMANN
Metabolism Branch,
National Cancer Institute,
Bethesda, Maryland 20014

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the supernatant after absorption. It was found that more than 98 percent of all concanavalin A had been removed by the absorption procedure. The immunological suppressive capacity of each concanavalin A supernatant was confirmed by measuring its ability to block pokeweed mitogen–stimulated immunoglobulin M (IgM) synthesis of normal mouse splenocytes, as assessed by cytoplasmic immunofluorescence at the termination of a 7-day culture period. Since each of these supernatants has a soluble immune response suppressor activity and is prepared according to the method used by Rich and Pierce to produce SIRS they are referred to by this acronym. It should be noted, however, that these are crude supernatants that contain other active factors. The SIRS was stored frozen in portions at −20°C until utilized. These frozen portions retained activity for at least 3 months under these storage conditions. The animals were treated twice weekly with 0.5 ml of the absorbed supernatants, thus representing one-half of the SIRS produced by 2×10^6 spleen cells exposed to concanavalin A and then cultured in 1 ml of media.

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* Present address: Arthritis Unit, Massachusetts General Hospital, Boston 02115.

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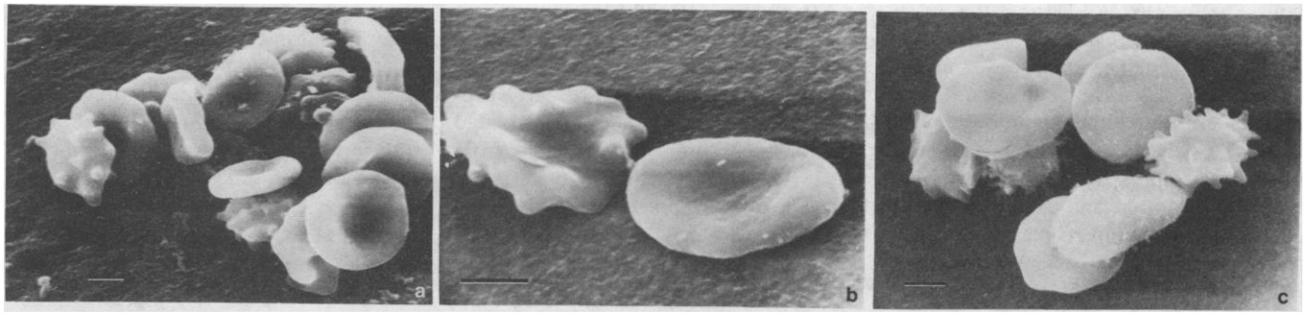
Morphologies of Cells from 1-Day Chick Embryos

Abstract. *Cells disaggregated from 1-day chick embryos show three morphologies, two reminiscent of mammalian erythrocytes and one epithelial. The presence of intermediate forms suggests that the three morphologies are interconvertible.*

During experiments in which we have been investigating the control of morphogenetic movements in early chick embryos by extracellular adenosine 3′,5′-monophosphate (cyclic AMP) signals, it became necessary for us to determine the shapes and sizes of cells disaggregated from the embryos (*1*). We examined the cells by light microscopy and scanning electron microscopy (SEM). We found that most cells were 3 to 5 μm in diameter and had one of three morphologies: biconcave, like mammalian erythrocytes; knobbed or hedgehog-like, a form also reported for mammalian erythrocytes (*2*); and finally epithelial-like, with projecting microvilli (see Fig. 1). They occurred in the following proportions: biconcave, 69 percent; knobbed, 18 percent; and epithelial-like, 13 percent. Intermediate forms were also seen, suggesting that the morphologies are interconvertible.

Scanning electron micrographs were taken of the cells and cell aggregates from disaggregated chick embryos after approximately 1 day's incubation [Hamburger-Hamilton, stage 5 (*3*, *4*)]. The entire blastodisks were removed and disaggregated by suspension in 0.1 m*M* EDTA in Ringer solution (*3*). After disaggregation, the cells were centrifuged and resuspended in Ringer solution twice at 4°C to remove all traces of EDTA. The cell suspensions were then plated onto 2 percent Difco purified agar made up in Ringer solution and allowed to settle for 2 hours at 37°C.

The cells on agar blocks (5 by 5 by 2.5 mm) were then prepared for SEM in a Coates & Welter model 50-4 scanning electron microscope. They were passed through a series of seven ethanol-H_2O mixtures ranging from 10 percent ethanol to absolute ethanol. Next they were transferred from absolute ethanol to amyl acetate in seven steps ranging from 10 percent amyl acetate in ethanol to 100 percent amyl acetate. Finally, they were dried by the critical-point method and



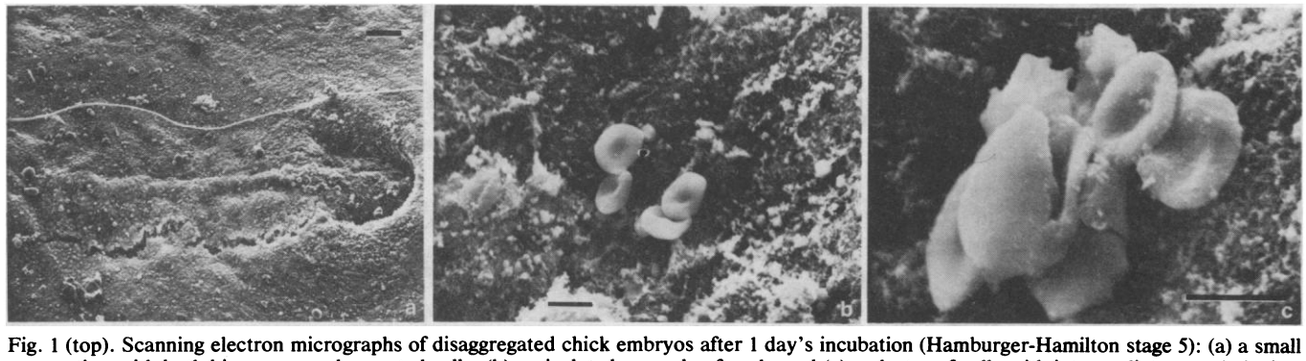


Fig. 1 (top). Scanning electron micrographs of disaggregated chick embryos after 1 day's incubation (Hamburger-Hamilton stage 5): (a) a small aggregation with both biconcave and crenated cells; (b) an isolated example of each; and (c) a cluster of cells with intermediate morphologies. Scale bars, 2 μm. Fig. 2 (bottom). Scanning electron micrographs of cell aggregates in whole embryo (Hamburger-Hamilton stage 7-8): (a) entire embryo (scale bar, 100 μm) and (b and c) cell aggregates in the area opaca with biconcave and crenated morphologies (scale bars, 6 μm).