Brief Definitive Report

ADMINISTRATION OF GROUP A STREPTOCOCCAL CELL WALLS TO RATS INDUCES AN INTERLEUKIN 2 DEFICIENCY

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In animal models of autoimmunity such as in NZB/NZW, and MRL/*lpr* mice, studies from several laboratories (1) have shown that spleen cells derived from these mice are deficient in the synthesis of IL-2. These observations have been extended to human autoimmune diseases; studies by Miyasaka et al. (2), Alcocer-Varela et al. (3) have shown a deficiency in the synthesis of IL-2 by peripheral blood lymphocytes obtained from patients with rheumatoid arthritis and systemic lupus erythematosus. Thus the inability of the cells of the immune system to synthesize IL-2 may contribute to the impaired immunoregulation and to the pathogenesis of these autoimmune disorders.

To determine the relationship of IL-2 to autoimmune diseases, we have examined the synthesis of this lymphokine in an animal model of rheumatoid arthritis. In this animal model, intraperitoneal administration of sonicated cell walls of group A streptococci induces a chronic erosive arthritis with histologic and radiologic features similar to human rheumatoid arthritis (4, 5). While this arthritis can be readily induced in the Lewis strain, studies by Wilder et al. (5) have shown that inbred Fischer rats are resistant to the development of the arthritic lesions. Using these resistant and susceptible strains, it was therefore possible to examine the relationship of IL-2 to clinical disease. This report describes these studies.

Materials and Methods

Inbred female Lewis (LEW/N) or Fischer (F344/N) rats (100 g), obtained from Charles River Breeding Laboratories, Wilmington, MA, were injected intraperitoneally with sonicated group A streptococcal cell walls (particle size <0.45 μm) suspended in PBS at a dose of 60 μg of cell wall rhamnose per gram body wt (4). Control rats received equivalent amounts of PBS. Rats were routinely observed for the development of clinical arthritis (hind paw inflammation). Hind paw diameters around the ankle joints were measured with a vernier caliper. At times indicated, control and cell wall–treated rats were sacrificed, and the hind paws were subjected to radiographic analysis. The spleens were removed, and single-cell suspensions were prepared. These spleen cells (4×10^5 cells) were cultured in microtiter wells for 72 h in 0.2 ml of completely supplemented RPMI-1640 medium containing various amounts of Con A. 18 h before harvest, these cultures were pulsed

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with 1 μCi of [^{3}H]thymidine, and the amount of cellular radioactivity was determined. All of these assays were run in triplicate.

For assays related to IL-2 synthesis, 5×10^6 cells/ml spleen cells were cultured in the above medium in the presence of 5 μg/ml of Con A. After 48 h, the medium was harvested and the IL-2 content was determined using an IL-2-dependent cell line (CTLL) using the procedure of Gillis et al. (6).

To examine the responsiveness of spleen cells to IL-2, 0.1 ml of spleen cell suspensions (4×10^6 cells/ml) derived from control and cell wall–treated rats were cultured for 72 h in the presence of IL-2 (100 U/ml) and various amounts of Con A (total volume, 0.2 ml). 18 h before harvest, the cells were pulsed with [^{3}H]thymidine (1 μCi). Cells were harvested and assayed for radioactivity.

To determine the expression of IL-2-R on spleen cells, a mouse mAb (ART-18) directed to rat IL-2-R was radiolabeled with ^{125}I as reported (7). Spleen cells from normal and cell wall–treated rats (5×10^6 cells/ml) were cultured for 48 h in the presence of Con A. Viable cells were harvested by centrifugation through a discontinuous Ficoll-Paque gradient (Pharmacia Fine Chemicals Piscataway, NJ). The cells were washed with PBS-BSA buffer containing 10 mM sodium azide. $1–5 \times 10^6$ viable cells in 0.1 ml of PBS-BSA were incubated with 1–2 μg of ^{125}I-labeled rat anti–IL-2-R antibody. After 60 min at 4°C, 1 ml of cold PBS-BSA was added, and the cells were pelleted by centrifugation. The pelleted cells were washed with 1 ml of cold PBS-BSA. After centrifugation, the pelleted cells were assayed for radioactivity.

Results

Confirming earlier observations of Wilder et al. (5), the Lewis strain of rats when treated with streptococcal cell walls developed clinical arthritis, whereas the Fischer strain of rats were resistant to the development of this lesion (results not shown).

Spleen cells derived from either Lewis or Fischer rats that had been treated with cell walls exhibited a decreased mitogenic response to Con A when compared to spleen cells derived from aged-matched control strains (Fig. 1, *A* and *B*). To determine whether exogenous IL-2 would normalize this depressed mitogenic response, spleen cells derived from cell wall–treated Lewis rats were cultured in the presence of IL-2 (100 U/ml) and various concentrations of Con A. Results shown in Table I indicate that the addition of exogenous IL-2 did not normalize the mitogenic response. These observations suggest that these spleen cells are either deficient in the expression of IL-2-R or that IL-2-R are not functional.

The ability of spleen cells to synthesize IL-2 was also examined. On day 1 after cell wall treatment, IL-2 synthesis by spleen cells derived from Lewis rats was similar to normal. However, with cell wall–treated Fischer rats, IL-2 synthesis by spleen cells was significantly lower than normals (Table II). On days 5, 19, and 33 after cell wall treatment, spleen cells from both Lewis and Fischer rats synthesized decreased amounts of IL-2.

The deficiency in splenic IL-2 synthesis induced by streptococcal cell walls was not due to the toxic effect of the cell walls on the spleen. For example, viable cell count in the spleen of normal Lewis rats was $240 \pm 22 \times 10^6$, whereas spleens of cell wall–treated rats (day 19) contained $168 \pm 16 \times 10^6$ cells. On day 33, spleens of normal Lewis rats contained $243 \pm 16 \times 10^6$ cells, whereas spleens of cell wall–treated rats contained $168 \pm 34 \times 10^6$ cells.

To determine whether treatment of rats with cell walls depleted spleens of lymphocytes of a specific phenotype, spleen cells from normal Lewis rats and those treated with cell walls (day 31) were analyzed by flow cytometry (FACS

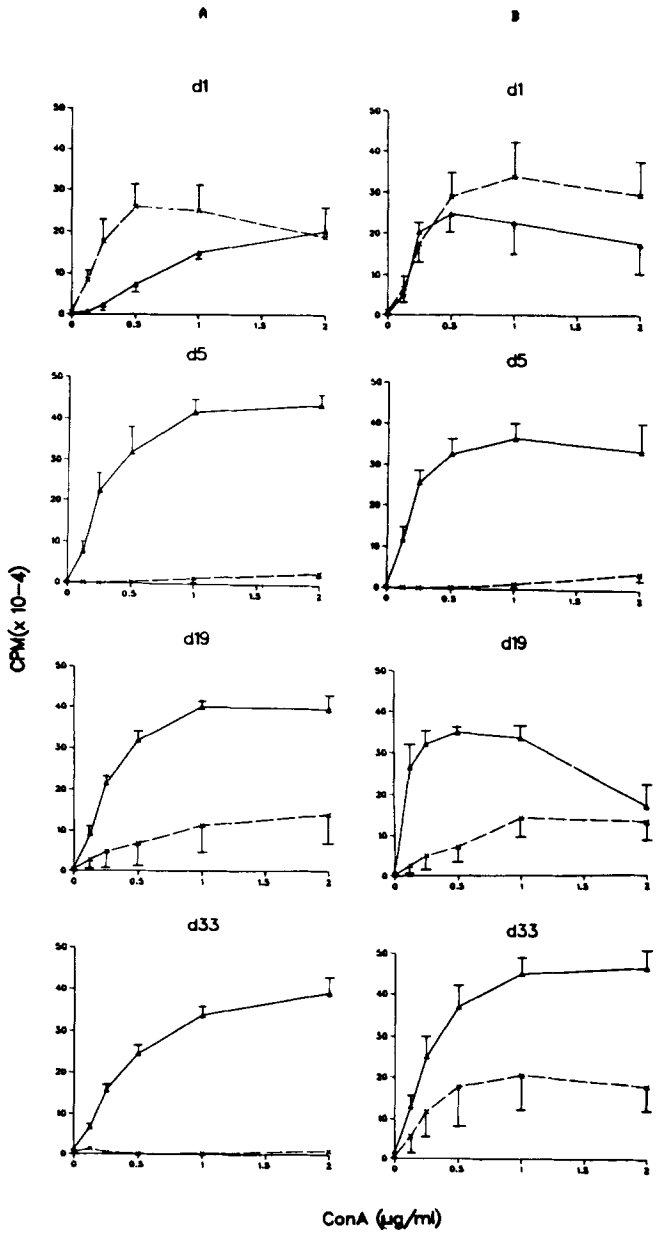

FIGURE 1. Mitogenic response of normal (◀----▶) and cell wall–treated (x-----x) Lewis (*A*) and Fischer (*B*) rats. Spleen cells were isolated on day indicated.

IV; Becton Dickinson Immunocytometry Systems, Mountain View, CA). The results of this analysis are shown in Table III. The percentage of cells bearing the surface marker W3/25 (helper/inducer subset) was similar in the spleens of normal and cell wall–treated rats. In rats, this subset of lymphocytes are responsible for the synthesis of IL-2. No significant differences between spleens of normal and cell wall–treated rats was detected when the cells were analyzed for

TABLE I

Effect of Exogenous IL-2 on Mitogenic Response of Spleen Cells Derived from Arthritic Lewis Rats

Con A concentration (μg/ml)	[^{3}H]Thymidine incorporation (cpm × 10^{-4})			
	Normal		Arthritic	
	No additions	Plus IL-2 (100 U/ml)	No additions	Plus IL-2 (100 U/ml)
0	0.5 ± 0.07	3.5 ± 0.4	0.1 ± 0	0.05 ± 0.03
0.05	11.1 ± 1.0	14.3 ± 1.6	0.3 ± 0.04	0.2 ± 0.09
0.1	22.3 ± 1.2	22.6 ± 2.1	0.4 ± 0.08	0.3 ± 0.2
0.5	32.4 ± 2.9	34.3 ± 3.1	0.5 ± 0.06	0.4 ± 0.2
1.0	40.2 ± 1.9	44.3 ± 2.3	0.3 ± 0.1	0.6 ± 0.4
2.0	40.6 ± 3.5	41.8 ± 3.6	0.5 ± 0.3	0.9 ± 0.7

Spleen cells from at least five animals were used for each assay on day 33 after cell wall treatment. Cultures were incubated for 72 h at 37°C. Other details of the assay are described in the text.

TABLE II

IL-2 Synthesis by Splenocytes Derived from Cell Wall–Treated Lewis and Fischer Rats

Days after cell wall treatment	IL-2 synthesis (U/ml supernatant)			
	Lewis		Fischer	
	Normal	Cell wall–treated	Normal	Cell wall–treated
1	289 ± 83.4 (6)*	391 ± 55.6 (6)	199 ± 32.2 (6)	76 ± 26.4‡ (6)
5	236 ± 52.0 (5)	28 ± 3.0‡ (5)	146 ± 41.0 (6)	41 ± 6.3‡ (6)
19	155 ± 62.1 (7)	23 ± 11.0‡ (7)	73 ± 14.2 (11)	9 ± 3.6‡ (11)
33	242 ± 47.3 (9)	34 ± 19.3‡ (9)	94 ± 13.8 (7)	36 ± 22.3‡ (7)

* Values in parentheses represent the number of animals from whom spleen cells were used for each assay.

‡ $p \leq 0.05$ as compared to the respective normal group.

TABLE III

FACS Analysis of Spleen Cells Derived from Normal Lewis Rats and Those Treated with Streptococcal Cell Walls

Rats	Percentage of cells staining for:			
	W3/25	OX-6	OX-8	IgG
Normal (n = 6)	37 ± 0.7	35 ± 0.8	17 ± 0.4	38 ± 1.1
Cell wall–treated (n = 5)	37 ± 3.1	20 ± 2.0*	21 ± 2.7	29 ± 2.5*

* FACS analysis performed 31 d after cell wall treatment. $p \leq 0.05$ as compared to normal rats. Other details of the assay are described in the text.

OX-8 (suppressor/cytotoxic subset). However, a significant decrease in cells staining for OX-6 (Ia^+) and IgG was observed in spleens of cell wall–treated rats. These observations indicate that a deficiency in the synthesis of splenic IL-2 after cell wall treatment is not due to a depletion of the helper/inducer subset in the spleen.

The conditions for the induction of this IL-2 deficiency were investigated. In results not shown, addition of various amounts of streptococcal cell walls to normal spleen cells did not inhibit Con A–dependent IL 2 synthesis. These observations indicate that in vivo processing of cell walls is required for the induction of IL-2 deficiency. To validate the observations that spleen cells from

TABLE IV

Binding of ^{125}I-labeled Anti-Il-2-RmAb to Con A-cultured Splenocytes Derived from Normal and Cell Wall-treated Rats

Rats	Antibody bound (ng/10^6 cells)
Normal Fischer	39.7 ± 3.5
Cell wall-treated Fischer	44.9 ± 5.1
Normal Lewis	36.7 ± 0.2
Cell wall-treated Lewis	37.8 ± 1.5

Cells were assayed 32 days after cell wall treatment. Spleen cells derived from these animals were cultured in Con A, and the binding assays were run in triplicate. Spleen cells from at least three animals were used for each assay. Other details are described in the text.

cell wall–treated rats were deficient in the synthesis of IL-2, several control experiments were conducted. Addition of various amounts of cell walls to cultures of CTLL cells did not inhibit their proliferative response to IL-2. Therefore, cell walls are not cytotoxic to CTLL cells. Spent medium from Con A–cultured splenocytes derived from cell wall–treated rats did not alter the proliferative response of CTLL cells to IL-2. Thus the spent medium does not contain an inhibitor that can interfere with IL-2 assays.

To assess the amount of IL-2-R on splenocytes derived from rats treated with cell walls, these cells and normal splenocytes were cultured in the presence of Con A. Viable cells were incubated with ^{125}I-labeled rat anti–IL-2-R antibody. Results shown in Table IV indicate that splenocytes derived from normal and cell wall–treated rats bind equivalent amounts of the labeled antibody. These results indicate that the IL-2-R expression on spleen cells of cell wall–treated rats is similar as in normal cells.

Discussion

Recent studies by Miyasaka, et al. (2) have shown a deficiency in the synthesis of IL-2 by peripheral blood lymphocytes and synovial mononuclear cells isolated from patients with rheumatoid arthritis. Since IL-2 plays a key role in the generation and maintenance of a normal immune response, a defect in its synthesis can contribute to the impaired immunoregulation observed in rheumatoid arthritis. Results reported in this communication indicate, for the first time, that administration of streptococcal cell walls to rats induces a splenic IL-2 deficiency. Since this deficiency is induced in arthritic-susceptible (Lewis) and resistant (Fischer) strains, it is likely that immunological abnormalities, in addition to IL-2 deficiency, are involved in the development of cell wall arthritis.

In murine strains of autoimmune disease (1), IL-2 deficiency is accompanied by a deficiency in IL-2-R. Using an immunological assay, the present studies indicate that, in cell wall–induced arthritis, IL-2-deficient spleen cells appear to express normal levels of surface IL-2-R.

Earlier studies by Hunter, et al. (8) have shown that cell-mediated immunity in rats with streptococcal cell wall arthritis is impaired. The mechanism(s) by which cell walls induced this deficiency was not known. The present studies indicate that streptococcal cell walls, in vivo, induce an IL-2 deficiency and this deficiency may, at least in part, contribute to the impairment in cell-mediated responses in these animals. The mechanism(s) by which streptococcal cell walls,

in vivo, induce an IL-2 deficiency has not been established. However, the observation that cell walls induce an IL-2 deficiency in a short time makes this animal model useful for studies related to immunoregulation. In addition, the model provides for an assay of potential therapeutic agents that may modulate IL-2 synthesis.

Summary

Intraperitoneal administration of group A streptococcal cell walls to Lewis rats induces a chronic arthritis, whereas the Fischer strain is resistant to the development of the lesion. Spleen cells from cell wall–treated rats (Lewis and Fischer) are deficient in the synthesis of IL-2. Using an mAb directed against the rat IL-2-R, the present studies indicate that the expression of IL-2-R on spleens of cell wall–treated rats is normal. However, the addition of exogenous IL-2 to spleen cells cultured with Con A does not stimulate the mitogenic response.

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