STREPTOCOCCAL CELL WALL ARTHRITIS

Passive Transfer of Disease with a T Cell Line and Crossreactivity of Streptococcal Cell Wall Antigens with *Mycobacterium tuberculosis*

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A severe polyarthritis can be induced in female Lewis rats by intraperitoneal administration of sonicated cell walls derived from group A, B, and C streptococci (1). This clinical disease is characterized by an acute phase, detected between days 1 and 4, and a chronic phase that occurs ~3 wk after immunization. Similarities exist between this disease in rats and human rheumatoid arthritis (RA).[1] As seen in RA, the incidence of cell wall-induced disease is greater in females than males; genetic factors play a key role in disease induction; the disease is complement dependent; the immune cells of cell wall-treated rats are IL-2 deficient; and the clinical disease is remittive and relapsing (2–4).

Although a large number of T lymphocytes are present in the inflamed synovium of patients with RA, their role in the pathogenesis of the disease is not entirely clear (5, 6). However, in animal models of arthritis, cell-mediated immunity to the inciting antigen appears to play a key role. Adjuvant-induced arthritis is a T cell–dependent lesion, and activated lymphocytes from adjuvant arthritic rats (7, 8) and a *Mycobacterium tuberculosis* (MT)-specific T cell clone (9) can passively transfer clinical and histological disease to naive syngeneic recipients. In addition, passive disease is not detected in rats treated with an anti-T cell antibody (10). Type II collagen-induced arthritis in rats can also be transferred to syngeneic animals by T cells (11, 12). The role of T cells in the pathogenesis of streptococcal cell wall (SCW)-induced arthritis has also been extensively studied (13–15), but evidence for their role in the disease has been indirect. It has been demonstrated that the acute phase of the disease is T cell independent and the development of the chronic phase is T cell dependent. In addition, the chronic phase of the disease does not develop in athymic rats or in those treated with cyclosporin A. Histological studies conducted in this model indicate that the synovial tissue of rats in the chronic phase of disease contains T cells (16). Nondigestible cell wall fragments persist in the synovial tissue, and it has been

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[1] *Abbreviations used in this paper:* MT, *Mycobacterium tuberculosis*; PE, phycoerythrin; RA, rheumatoid arthritis; SCW, streptococcal cell wall.

suggested that the continued presence of antigen in the inflamed synovium may, at least in part, account for the presence and proliferation of activated T cells in the synovium and for the chronicity of disease (16).

In the present studies, we have been successful in developing two SCW-specific T cell lines from lymph nodes of SCW arthritic rats that can transfer disease to irradiated recipients. Passively transferred disease was chronic but less severe than active disease and was characterized by synovial lining proliferation, cellular infiltration in the subsynovial tissue, fibrin deposition in the joint space, pannus formation, and marginal erosions of the bone and cartilage. These observations provide direct evidence for the participation of T cells in the pathogenesis of SCW arthritis. Both T cell lines (SCW 100 and SCW 103) also proliferated in response to sonicated and heat-killed MT, an antigen that is active in inducing adjuvant arthritis. These latter observations indicate that there is antigenic crossreactivity between SCW and MT and suggest a similarity in the etiopathogenesis of SCW and adjuvant arthritides. It is possible that an epitope(s) shared by these two antigens is sufficient to activate the T cell line for disease induction.

Materials and Methods

Induction of Active Arthritis. Cell walls were prepared from group A streptococci as previously described (1). Inbred female Lewis rats weighing ~100 g were obtained from Charles River Breeding Laboratories, Wilmington, MA. Arthritis was induced by intraperitoneal injection of sonicated SCW (size, <0.45 μ) at a dose of 10–15 μg rhamnose/g body weight. Rats were killed during the chronic phase of the disease. Adjuvant arthritis was induced in inbred male Lewis rats by immunization with an emulsion of heat-killed MT cells (virulent strain C, DT, and P; 121°C for 15 min) in IFA by methods described by Sloboda et al. (17). The MT antigen was prepared as described below.

Antigens Used for T Cell Proliferation. Sonicated SCW were prepared as described (1). Heat-killed MT was suspended in water and sonicated for 60 min. The sonicated preparation was centrifuged at 15,000 *g* and the supernatant was filtered through a 0.45-μ filter. The filtered material was used in the cell proliferation assays as described. An aliquot of this material was dried by lyophilization and weighed. *Escherichia coli* lysates (constructs JM105 and N4830) containing the recombinant 65-kD MT protein was generously provided by Dr. Thomas Shinnick, Centers for Disease Control, Atlanta, GA.

Establishment of T Cell Lines. The T cell lines were established essentially as described in earlier studies (18, 19). Lymph node cells derived from rats with chronic SCW arthritis were cultured in the presence of SCW antigen containing 10–20 μg/ml of cell wall-derived rhamnose. Cells were plated (0.2 ml) at a concentration of 5×10^5/well in 96-well microtiter plates in RPMI 1640 medium (Gibco Laboratories, Grand Island, NY) supplemented with nonessential amino acids, sodium pyruvate, Hepes, penicillin-streptomycin, 2-ME (5×10^{-5} M), and 1% autologous rat serum (proliferation media). At the end of a 3-d incubation, T cell blasts were isolated from the culture by Ficoll-paque (Pharmacia Fine Chemicals, Piscataway, NJ) separation techniques. Isolated T cell blasts were cultured in U-bottomed 96-well microtiter trays at a concentration of 10^4 cells/well in RPMI supplemented with nonessential amino acids, sodium pyruvate, Hepes, penicillin-streptomycin, 2-ME, 5% FCS, and 10% Polyclone (Collaborative Research, Lexington, MA) (propagation media). Cells were split and fed with fresh propagation media every 3–4 d. After 7–8 d, 4×10^4 T cells/well were restimulated for 3 d with antigen in the presence of 5×10^5 irradiated (1,500 rad) syngeneic spleen cells. These T cell lines were maintained in culture by alternating antigen stimulation cycles with cycles in propagation media.

A control T cell line reactive to OVA was also established from lymph node of rats immunized with OVA by using the procedures outlined above.

Proliferation Assays. To determine cell reactivity to different antigens, primary lymph node cells derived from SCW arthritic rats and adjuvant arthritic rats and the SCW-specific T cell line were incubated with various concentrations of the antigens in proliferation media. Lymph node cells were cultured in 0.2 ml medium at 5×10^5 cells/well with the appropriate dilution of antigen. The cells were pulsed on day 3 with 1 μCi [^{3}H]thymidine, harvested on day 4, and the amount of radioactivity incorporated was determined using a Betaplate flat bed liquid scintillation system (LKB Instruments Inc., Gaithersburg, MD). For the T cell line proliferative assays, each microtiter well contained 4×10^4 T cells, 5×10^5 irradiated syngeneic spleen cells (1,500 rad), and various concentrations of antigen. The volume in each microtiter well was 0.2 ml. After 3 d of incubation, the cells were pulsed for 6 h and the amount of radioactivity was then measured. All samples were done in triplicate.

Passive Transfer of Arthritis. At the end of the antigen stimulation cycle of the T cell lines (SCW 100 and SCW 103), blasts were recovered by Ficoll-paque centrifugation, washed extensively, and resuspended in PBS. Irradiated (600 rad) inbred female Lewis rats were injected with $7.5–15 \times 10^6$ SCW-specific T cells intravenously. Rats were monitored for clinical disease by measuring hind paw diameters (around the ankle joint) with vernier calipers. The hind paws were saved for histological and immunohistochemical analysis.

Histology. Recipient rats were killed on day 7, 14, or 38 after cell transfer and the hind paws were skinned and immersed in demineralizing solution for 6–8 wk (20). At the end of the demineralization process, paws were coated with OCT tissue embedding media and frozen by immersion in a dry ice and isopentane bath. The embedded tissue was stored at −70°C. 7–10-μm sections of frozen tissue were prepared on a cryostat (Bright Instrument Co., Huntingtdon, UK) and the sections were melted onto gelatinized glass slides. The slides were allowed to air dry for 1–2 h at room temperature. The sections were fixed in acetone for 10 min at room temperature and stored at −20°C. The frozen sections were stained with Diff-Quik stain (American Scientific Products, McGaw Park, IL) and scored for synovial lining hyperplasia, cellular infiltration, deposition of cells and fibrin in the synovial space, pannus formation, cartilage destruction, and marginal bone erosions (21). The subjective scores ranged from 0 to 4; 0 being normal and 4 being extensive changes.

Immunocytochemistry. Immunocytochemical staining was performed as previously described (21). Briefly, frozen tissue sections were incubated in a stepwise fashion with mAbs, sheep anti-mouse IgG combined with normal rat serum, mouse monoclonal peroxidase antiperoxidase, and finally, the substrate, 3,3-diaminobenzidine, $NiCl_2$, and H_2O_2. Between each step the samples were washed with Tris buffer (50 mM Tris, 0.15 M NaCl, pH 7.6). The sections were counterstained with methyl green and mounted in Permount (Fisher Scientific Co., Pittsburgh, PA).

The following mouse anti-rat mAbs were used: W3/13 (T cells and polymorphonuclear cells); W3/25 (Th cells and macrophages); OX6 (Ia); OX8 (Ts/cytotoxic cells and NK cells). These antibodies were purchased from Accurate Chemical & Scientific Corp., Westbury, NY). Other mAbs used included OX19 (T cells) and ED1 (macrophages). These antibodies were obtained from Bioproducts for Science, Inc., (Indianapolis, IN). The extent of infiltration by cells of a particular phenotype was scored subjectively (21) using a scale of 0 to 4; normal tissue was scored 0 and extensive infiltration by a particular cell phenotype was scored 4.

Cell Staining and FACS Analysis. OX19, OX8, W3/25, OX6, and anti-κ were bioinylated as described (22). FITC-conjugated W3/25 was purchased from Accurate Chemical and Scientific Corp. OX39 (Bioproducts for Science, Inc.) was used as an unconjugated antibody. $1–3 \times 10^5$ cells were incubated with diluted biotinylated mAbs for 20 min on ice. The cells were washed twice with PBS containing 2% FCS and 0.01% NaN_3 and were then incubated with strepavidin-phycoerythrin (PE; Becton Dickinson & Co., Mountain View, CA) for 20 min. The cells incubated with unconjugated antibody OX39 were incubated with FITC-goat anti-mouse IgG (Accurate Chemical and Scientific Corp.) that had been preabsorbed on a rat IgG-sepharose column. Finally, the cells were washed twice with PBS/NaN_3 and fixed in 1% paraformaldehyde. For double-color staining, the first antibody used was biotinylated OX19 and strepavidin-PE, followed by incubation with FITC-W3/25. Analysis was conducted by flow cytometry (FACS IV System; Becton Dickinson & Co.).

Results

T Cell Line: Phenotypic Analysis and Proliferative Response to SCW. Earlier studies from this laboratory and from others (13–15) have shown that the chronic phase of SCW arthritis cannot be induced in athymic rats and is therefore T cell dependent. To examine the role of T cells in the induction of SCW arthritis, a T cell line was developed from the lymph nodes of rats during the chronic phase of SCW arthritis. The proliferative response of this T cell line (SCW 100) to various concentrations of SCW (rhamnose equivalents) is shown in Table I. SCW 100 expressed pan T surface markers OX19 and W3/13, Th surface marker W3/25, and was IL-2-R positive (OX39). A typical phenotypic analysis of this T cell line is shown in Table II.

Passive Transfer of Arthritis with T Cell Lines SCW 100 and SCW 103. To determine the T cell contribution to the pathogenesis of this disease, the T cell lines were stimulated with SCW, and varying amounts of stimulated cells were transferred intravenously to naive syngeneic irradiated (600 rad) recipients. Recipient rats were monitored for clinical disease. Passive transfer resulted in clinical disease in 12/12 recipient rats with an onset between days 5 and 11 after cell transfer (Table III). As a quantitative measurement of clinical disease, hind paw diameters of the recipient rats were measured periodically throughout the course of the experiment. As shown in Table IV, rats with passive SCW arthritis exhibited significantly greater hind paw diameters

TABLE I

Dose-Response Effect of Streptococcal Cell Wall Antigens on the Proliferation ([^{3}H]Thymidine Incorporation) of the T Cell Line (SCW 100)

Antigen concentration	Proliferation	Stimulation index
μg rhamnose equivalents/ml	*mean cpm ± SEM*	
0.0	769 ± 152	1
1.25	35,478 ± 2,123	46
2.5	46,053 ± 1,780	60
5.0	60,369 ± 3,664	79
10.0	63,803 ± 4,643	83
20.0	46,283 ± 2,122	60

All assays were conducted in triplicate. Other details of the assay are described in the text.

TABLE II

Phenotypic Analysis of the T Cell Line SCW 100

Primary mAb	Percent positive cells*
OX-19$^+$ (T cells)	93.4
W3/13$^+$ (T cells)	84.3
W3/25$^+$ (Th cells and macrophages)	90.0
OX-19$^+$; W3/25$^+$ (Th cells)	78.5
OX-39$^+$ (IL-2R$^+$ cells)	83.0
OX-8$^+$ (Ts/cytotoxic cells)	2.1
OX-6$^+$ (Ia$^+$ cells)	2.0
Anti-κ^+ (B cells)	0.0

* Analysis conducted by flow cytometry. Details of the assay are described in the text.

TABLE III

Passive Transfer of Arthritis to Recipients Using the T Cell Line SCW-100: Disease Incidence

Experiment	No of cells transferred*	Disease incidence	Day of onset
	$\times 10^6$		
1	8–10	3/3	5–6
2	7.5	1/1	11
3	10	1/1	5
4	10	2/2	7–9
5	10–15	5/5	6–10

* Cells were injected intravenously into irradiated female recipient (600 rad) Lewis rats on day 0. Recipients were monitored for clinical disease by measuring hind paw diameters with a vernier caliper. Other details of the assay are described in the text.

TABLE IV

Passive Transfer of Arthritis with the T Cell Line SCW-100: Time Course of Clinical Disease Development in the Recipient

Day of measurement	Hind paw diameters	
	Injected recipients	Control rats
	mm ± SEM	
5	6.7 ± 0.07* (12)	6.2 ± 0.07 (5)
7	7.1 ± 0.07* (12)	6.4 ± 0.06 (5)
9	7.3 ± 0.08* (9)	6.4 ± 0.04 (4)
14	7.3 ± 0.06* (3)	6.4 ± 0.08 (4)
26	7.2 ± 0.12* (3)	6.4 ± 0.06 (2)
38	7.0 ± 0.2* (3)	6.4 ± 0.05 (2)

Recipient rats received 7.5–15 × 10^6 cells i.v. Values in parentheses represent the number of rats used.

* Statistical significance, $p \leq 0.0005$, as compared with control rats. Other details of the assay are described in the text.

as compared with naive controls. Three recipient rats were monitored for disease up to 38 d after cell transfer and all exhibited chronic disease. Both the front and hind paws were involved. The passive disease, however, was not as severe as the active disease. Hind paw diameters of animals with active SCW arthritis were generally larger than those with passive disease. In one representative experiment, average hind paw diameters in animals with active arthritis were 7.9 ± 0.1 mm (n = 18), as compared with those with passive arthritis where the mean paw diameters were 7.2 ± 0.1 mm. Hind paw diameters of untreated control rats were 6.4 ± 0.1 mm. In results not shown, naive irradiated recipients injected intravenously with lethally irradiated T cells did not exhibit the clinical lesion. Similar to the T cell line SCW 100, the second T cell line, SCW 103, was equally active in transferring clinical arthritis to naive irradiated syngeneic recipients (results not shown).

Histological Evaluation of the Arthritic Lesion in the Hind Paws. The histological changes that accompany the chronic phase of active SCW arthritis include hyperplasia of the synovial lining cells, cellular infiltration in the subsynovial tissue, pannus formatin, cartilage destruction, and bone erosions (1). The hind paws of eight animals

with passive SCW disease were sectioned, stained with Diff-Quik, and analyzed for histological changes. The results are summarized in Table V. Synovial tissue in normal rat paws is composed of a compact layer of synoviocytes that lie adjacent to adipose and connective tissue (Fig. 1 *A*). Paws from six rats killed between days 7 and 14 after cell transfer showed hyperplasia of synovial lining cells, infiltration of the adjoining tissue by macrophages, lymphocytes, and PMNs (Fig. 1, *B* and *C*), and some areas of marginal bone erosions (Fig. 1 *D*). Three of six rats had significant deposition of fibrin and cells in the joint spaces (Fig. 1 *B*). While there was clear demonstration of hypertrophy of synovial villi and hyperplasia of the tissue adjacent to the synovial cells, a pannus was not detected at days 7 and 14 after cell transfer. Two hind paws sectioned at day 38 after cell transfer had all of the above changes. In addition, cartilage destruction and a pannus were detected in one rat at 38 d after cell transfer.

Immunocytochemical Analysis of the Cellular Infiltrate in the Arthritic Lesion. To further characterize this passive arthritis induced by the T cell line, SCW 100, immunocytochemical staining methods were used to determine the phenotype of the cellular infiltrate. Demineralized paw sections were stained with mAbs OX6 (Ia), OX19 (T cells), W3/13 (PMNs and T cell), ED1 (macrophages), and OX8 (Ts/cytotoxic and NK cells). The results of this analysis are shown in Table VI. Normal synovial lining cells and adjacent adipose tissue contained a small number of Ia^+ $ED1^+$ cells (Fig. 1 *E*). In animals with passive disease, there was an explosive infiltration of large Ia^+, $ED1^+$ cells in the tissue adjoining the synovium and in areas of marginal bone erosions (Fig. 1, *F*, *G*, and *H*). These large Ia^+, $ED1^+$ cells were the predominant cell type in the infiltrate and are presumably macrophages (Fig. 2 *A*). In addition to the Ia^+ cells found in the infiltrate, proliferating synovial lining cells showed enhanced expression of Ia as compared with controls. $OX19^+$ T cells were present in the tissue of arthritic animals but not in controls. There were fewer $OX19^+$ T cells infiltrating the tissue than there were macrophages (Fig. 2 *B*). $W3/13^+$ cells were also found in the arthritic lesion and were not seen in sections of control animals.

TABLE V

Histological Analysis of the Hind Paws of Recipient Rats that Had Received the T Cell Line SCW-100

			Histological grade					
Animal	Day of disease onset	Day of sacrifice	Synovial lining hyperplasia	Cellular infiltrate	Fibrin and cells in the joint space	Pannus	Bone erosions	Cartilage destruction
1	5	7	1.0	1.5	0	0	1.0	0
2	5	7	2.0	1.0	0	0	1.0	0
3	5	7	2.0	3.0	3	0	4.0	1
4	6	14	3.0	3.0	2	0	2.0	0
5	7	14	2.5	1.0	0	0	2.0	0
6	7	14	1.5	1.0	1	0	1.0	0
7	7	38	3.0	3.5	0	1	4.0	2
8	7	38	3.0	3.5	0	1.5	3.0	1

Recipient rats were injected with $7.5\text{-}15 \times 10^6$ cells and the rats were killed on days indicated. Paw sections were stained and the sections were graded for the various parameters listed. Grade 0, normal and grade, 4 indicates extensive changes. The grades for a normal paw was 0 for all of the parameters listed. Other details of the assay are described in the text.

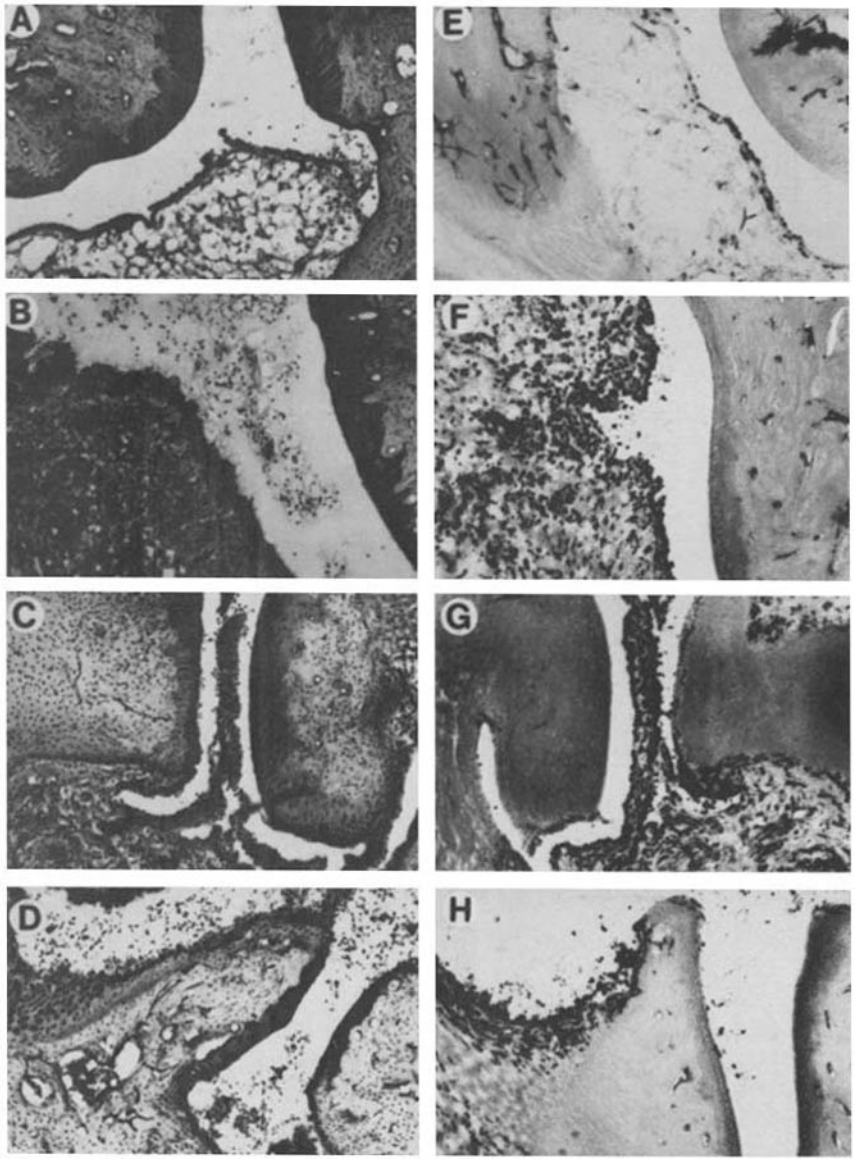

FIGURE 1. Histological and immunocytochemical analyses of hind paws of normal rats and rats with passive SCW arthritis. *A–D* were stained with Diff-Quik. *A* shows synovial tissue and adjacent bone and cartilage in a normal rat paw. *B–D* are sections from arthritic rats and show cellular infiltration of subsynovial tissue (*B*, *C*), cellular and fibrin deposition in joint spaces (*B*, *D*), and marginal bone erosion (*D*). In *E–H*, similar sections were stained for Ia^+ cells using mAb OX6 (× 125).

W3/13 is a pan-T cell marker that crossreacts with PMNs. Morphologically, the majority of these $W3/13^+$ cells had a multilobed nucleus and were most likely PMNs (Fig. 2 *C*). Also found in the arthritic lesion were $OX8^+$ cells (Fig. 2 *D*). This mAb binds to both NK cells and T s/cytotoxic cells. These cells were not present in the

TABLE VI

Immunohistochemical Evaluation of the Cells Present in the Synovium of the Paws of Rats that Were Treated with the T Cell Line SCW-100

Animal	Day of disease onset	Day of sacrifice	Phenotype of cells infiltrating the synovium*				
			OX-6	ED1	W3/13	OX-19	OX-8
1	5	7	2	2	2	3	1
2	5	7	4	4	3	3	3
3	5	7	4	4	3	2	2
4	6	14	3	3	2	2	1
5	7	14	2	1.5	1	0.5	0.5
6	7	14	2	2	1	1	0.5

Recipient rats were injected with 7.5–15 × 10^6 cells. Other details of the assay are described in the text.

* Subjective scoring system is based on a scale of 0–4. Normal rat paw sections score 0 in all of the above categories.

demineralized paw sections derived from control animals. In results not shown, W3/25, which stains helper T cells and macrophages, gave results similar to those obtained with OX6 (anti-Ia).

Crossreactivity of Primary Lymph Node Cells from Adjuvant Arthritic Rats with SCW and of Lymph Node Cells from SCW Arthritic Rats with MT. The development of adjuvant

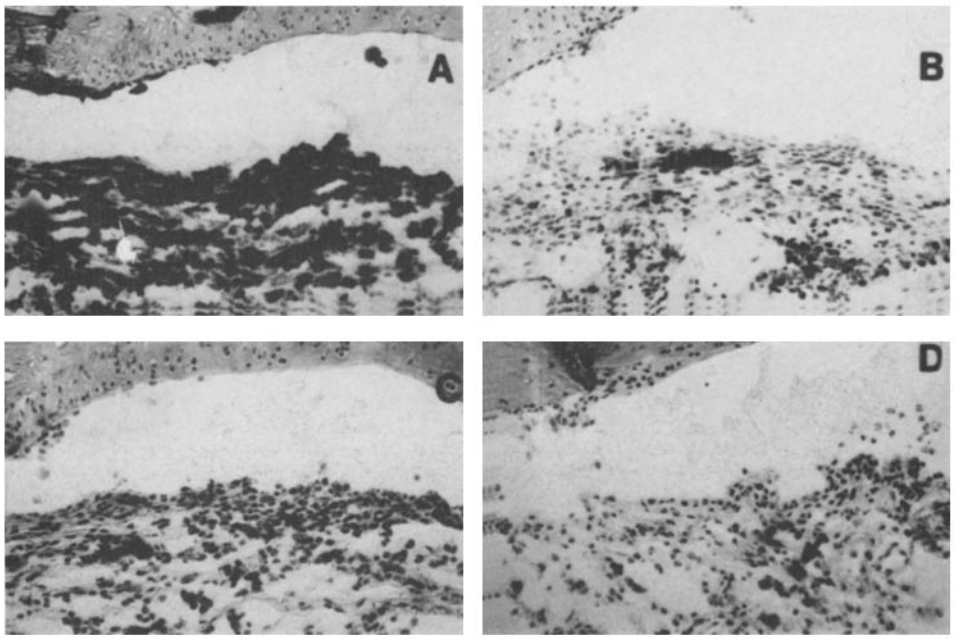

FIGURE 2. Immunocytochemical analyses of synovium in hind paws of rats with passive SCW arthritis. The section in *A* was stained for Ia^+ cells using mAb OX6. *B* is a representative area of synovial tissue stained for $OX19^+$ T cells. *C* was stained with mAb W3/13, which detects both T cells and PMNs. In *D*, the sections were stained with OX8, an mAb that stains both T s/cytotoxic cells and NK cells (× 200).

arthritis in rats is dependent on immunization of naive rats with cell walls derived from MT. Unlike the SCW-induced disease, induction of adjuvant arthritis requires intradermal administration of the heat-killed MT as an emulsion in incomplete adjuvant. To investigate any possible correlation between SCW and adjuvant arthritis, we have investigated the crossreactivity between these two bacterial antigens. Primary lymph node cells from animals immunized intraperitoneally with SCW or intradermally with MT in IFA were cultured with various dilutions of SCW or MT. As expected, lymph node cells derived from adjuvant arthritic rats (immunized with MT) proliferated to MT in a dose-responsive fashion (Table VII). These cells also proliferated to SCW, although the magnitude of the response was somewhat lower than that observed with MT. Thus, these cells were recognizing epitopes common to both SCW and MT. Primary lymph node cells derived from SCW arthritic animals were also examined for their proliferative response to SCW and MT. These cells proliferated in the presence of both antigens, albeit the response to MT was generally lower than that observed with the SCW antigen (Table VII).

Crossreactivity of the T Cell Line SCW100 with MT and with a 65-kD Recombinant Mycobacterium Protein. Since crossreactivity between the two antigens was demonstrated by using primary lymph node cells, it was of interest to determine whether the T cell line that was active in transferring passive SCW arthritis would also exhibit antigenic crossreactivity. The results shown in Table VIII show that this arthritogenic T cell line proliferated vigorously in the presence of either SCW or MT antigens. A similar proliferative response to SCW and MT antigen was detected with the other T cell line (SCW 103).

TABLE VII

Proliferative Response of Primary Lymph Node Cells Isolated from Adjuvant or SCW Arthritis

Antigen	Concentration	Proliferation of SCW lymph node cells	SI	Proliferation of adjuvant arthritis lymph node cells	SI
	μg rhamnose/ml	*mean cpm ± SEM*		*mean cpm ± SEM*	
SCW	20	23,333 ± 216	7.5	19,101 ± 3,596	14.7
	10	21,731 ± 2,741	7.0	11,870 ± 119	9.1
	5	26,752 ± 7,817	8.6	10,953 ± 922	8.4
	2.5	22,565 ± 2,077	7.3	10,329 ± 1,498	8.0
	1.25	21,407 ± 2,791	6.9	7,371 ± 2,099	5.7
	μg dry weight/ml				
MT	50	14,864 ± 1,784	4.8	36,809 ± 7,293	28.3
	10	13,696 ± 1,075	4.4	27,887 ± 4,590	21.5
	5	16,292 ± 2,071	5.3	28,570 ± 3,754	22.0
	1	9,522 ± 839	3.1	21,812 ± 5,099	16.8
	0.5	7,174 ± 1,503	2.3	14,794 ± 1,073	11.4
	0.1	4,959 ± 2,753	1.6	4,099 ± 469	3.2
None		3,105 ± 321		1,299 ± 59	

5×10^5 lymph node cells from rats with chronic SCW or adjuvant arthritis were cultured with various amounts of antigen as indicated. On day 3, the cells were pulsed with [^{3}H]thymidine for the last 20 h of incubation. The cells were harvested and analyzed for incorporation of radioactivity. All samples were done in triplicate. SI, stimulation index.

TABLE VIII

Proliferation of the T Cell Line SCW-100 to Streptococcal Cell Wall Antigens and to Mycobacterium

Antigen	Concentration	Proliferation		
		Exp. 1	Exp. 2	Exp. 3
	μg rhamnose equivalents/ml		*mean cpm ± SEM*	
Streptococcal cell walls	20.0	76,333 ± 10,468	119,604 ± 6,869	55,170 ± 4,522
	10.0	82,670 ± 10,184	133,664 ± 8,399	37,633 ± 2,745
	5.0	72,556 ± 4,475	104,096 ± 10,309	36,717 ± 3,668
	2.5	80,258 ± 5,871	ND	27,086 ± 1,505
	1.25	55,140 ± 2,592	ND	25,514 ± 2,260
	μg dry weight/ml			
Mycobacterium	50.0	100,689 ± 9,107	31,539 ± 5,295	29,381 ± 2,393
	25.0	86,921 ± 4,986	60,522 ± 1,715	ND
	10.0	77,027 ± 8,494	44,856 ± 4,635	10,907 ± 858
	5.0	62,253 ± 5,735	25,481 ± 2,752	8,844 ± 1,057
	1.0	30,274 ± 2,713	8,523 ± 1,119	2,401 ± 116
None	-	3,541 ± 314	918 ± 93	589 ± 38

4×10^4 T cells were cultured with various amounts of antigen as indicated in the presence of 5×10^5 irradiated syngeneic spleen. On day 3, cultures were pulsed with [^{3}H]thymidine for 6 h, harvested, and analyzed for radioactivity. Other details of the assay are described in the text.

The proliferative response of the SCW 100 to a recombinant 65-kD MT protein (23) was also investigated. This 65-kD protein has a 97% homology to the 65-kD protein of *M. bovis* and contains the amino acid sequence TFGLQLELT. Previous studies by van Eden et al. (24) have shown that this nonapeptide is recognized by the arthritogenic T cell clone (A2b) derived from rats with adjuvant arthritis and can induce tolerance to adjuvant arthritis. In addition, some sequence homology between the nonapeptide and cartilage proteoglycan has been shown (24). To determine whether the SCW 100 T cell line would recognize this nonapeptide sequence, proliferative studies were conducted with the recombinant 65-kD MT protein. In results not shown, the T cell line failed to proliferate to two *E. coli* lysates containing the 65-kD protein (JM105 and N4830). At a protein concentration of 10 μg/ml, the stimulation index in the presence of these lysates was 0.9 and 1.1, respectively.

OVA-specific Control T Cell Line. An OVA-specific T cell line was tested for its ability to proliferate to the various antigens tested above. This T cell line proliferated well to OVA (a stimulation index of 4.3 at 10 μg/ml) but failed to respond to SCW or MT antigens.

Discussion

T cell lines or clones have been useful in understanding the immunologic basis of autoimmune diseases in animal models. For example, an MT-specific T cell clone derived from adjuvant arthritic rats can induce passive arthritis in recipient rats (9). Type II collagen arthritis in mice and rats can be transferred to recipients with T cells or T cell clones (11, 12), indicating the importance of T cells in the development of this disease. Passive transfer of SCW-induced arthritis had not yet been accom-

plished. In the present studies a helper T cell line isolated from the lymph nodes of arthritic rats was able to transfer disease to recipient rats. The passive disease was chronic in nature but less severe than the active disease and was characterized by synovial lining hyperplasia, cellular infiltration and fibrin deposition in the subsynovial tissue and joint space, presence of a pannus, and marginal erosions of bone and cartilage. T cells, macrophages, and neutrophils were detected in this lesion. These observations indicate that T cells play an important role in the pathogenesis of SCW arthritis.

Another important observation presented in this study is the observation of antigenic crossreactivity between components of SCW and components of the arthritogenic MT preparations. Primary lymph node cells isolated from rats with SCW-induced arthritis or T cell lines (SCW 100 and 103) proliferate in the presence of MT antigen. However, SCW 100 did not proliferate to a recombinant 65-kD Mycobacterium protein (23) that contains the sequence TFGLQLELT. Thus, epitopes other than those present in the 65-kD protein but shared by MT and SCW antigens may be responsible for the proliferative response reported in these studies. For example, biochemical studies of Kotani et al. (25) of various cell wall structures clearly demonstrate that the respective peptidoglycan cores of SCW and MT are antigenically quite similar and could account for the observed reciprocal proliferation by the T cells. In addition, Lactobacillus cell walls, known to induce arthritis in rats (26), have a similar peptidoglycan structure. The fact that MT-specific T cell clones crossreact with cartilage proteoglycans provides the link from the bacterial antigen to the relevant mammalian antigens (27).

Although there has been no evidence that a single infectious agent is responsible for the pathogenic events that occur in RA, it has been suggested that bacteria may play a role in the disease process (28). This disease is known to be associated with a higher immune response to both streptococcal and mycobacterial antigens. For example, sera of patients with RA contain elevated levels of antibodies to streptococcal antigens (29); lymphocytes from some patients exhibit cell-mediated immune responses to mycobacterial antigens (30); and a high incidence of arthritis has been detected in patients undergoing cancer chemotherapy with Bacille Calmette-Guérin (BCG) (31). In the passive arthritis demonstrated in these studies and in other models (9, 11, 12), exogenous antigen is not required. The only prerequisite for arthritis induction in these animal models is that the T cells be sensitized to the inciting antigen (in vivo) before they are administered to recipients. The concept of molecular mimicry postulates that some antigenic components of bacteria share close antigenic similarity to certain tissue antigens of human origin and thereby are mimicking the antigen of human tissue (32, 33). If T cells that respond to the bacterial antigen also proliferate in response to a crossreactive host-derived antigen (molecular mimicry), the disease can be initiated. The MT-specific T cell clone that transfers passive arthritis also proliferates in the presence of cartilage proteoglycans, suggesting antigenic similarity between these two components (27). These observations provide some evidence for antigenic crossreactivity between bacterial and host antigens. We are currently investigating the response of our arthritogenic T cell lines (SCW 100 and SCW 103) to various bacterial (Lactobacillus cell walls, MDP, LPS) and host antigens (type I and II collagen; cartilage proteoglycans) and for the ability of the cell line to passively transfer disease after activation with one or all of these antigens. In addition,

attempts are being made to clone this T cell line. Using arthritogenic clones, experiments will be conducted that will measure the reactivity of these clones to various antigens of bacterial and host origin. Hopefully, such studies will provide additional information relevant to a bacterial etiology for the pathogenesis of human RA.

Summary

Primary lymph node cells derived from streptococcal cell wall arthritic rats or those derived from adjuvant arthritic rats proliferated in response to cell wall antigens derived from either streptococcal cell walls or those from *M. tuberculosis*. In addition, two T cell lines have been isolated from lymph nodes of rats during the chronic phase of streptococcal cell wall arthritis. These T cell lines transfered clinical disease to naive syngeneic irradiated recipients, and they proliferated in the presence of cell wall antigens derived from streptococci or antigens derived from Mycobacterium but failed to proliferate in the presence of the 65-kD antigen (containing the sequence TFGLQLELT) derived from Mycobacterium. These observations indicate that T cells play a crucial role in the pathogenesis of streptococcal cell wall arthritis and suggest that antigenic crossreactivity exists between cell walls of group A streptococci and antigens derived from Mycobacterium. The 65-kD Mycobacterium protein is not involved in the observed antigenic crossreactivity.

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