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Flare-up reaction of streptococcal cell wall induced arthritis in Lewis and F344 rats: the role of T lymphocytes

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SUMMARY

One i.p. injection of a sterile suspension of streptococcal cell walls (SCW) induces chronic erosive polyarthritis in susceptible Lewis rats, but not in resistant F344 or nude Lewis rats. Because continuous exacerbations may be one possible mechanism underlying chronic disease, we studied the mechanism of these flare-up reactions in Lewis and F344 rats. Injection of SCW into the right knee joint of rats induced a transient monoarthritis in both strains. Reactivation of the subsided arthritis by i.v. administration of the same antigen could be evoked only in the Lewis rat. Even repeated i.v. challenges with SCW failed to induce a flare-up reaction in the F344 rat, while the Lewis rat went through an exacerbation after every challenge. Removal of T lymphocytes by monoclonal antibodies before induction of an exacerbation rendered Lewis rats refractory to flare-up reactions, thus indicating the T cell-dependence of this reaction. Furthermore, when cell walls from heterologous bacteria were tested for their capacity to induce exacerbations of SCW-induced monoarthritis and to induce proliferation of SCW-specific T lymphocytes *in vitro*, a strong correlation between both features was found, again pointing to a role for SCW-specific T cells in exacerbations. Together, these data support our hypothesis that chronic arthritis is the result from repeated reactivations of a waning arthritis which are dependent on antigen-specific T lymphocytes.

Keywords streptococcal cell wall arthritis flare-up susceptibility T cells

INTRODUCTION

Despite a wide array of studies in various animal models, the pathogenesis of different forms of chronic joint inflammation still remains to be elucidated. Two extensively used models for studying arthritis are antigen-induced arthritis (AIA) in mice (van den Berg *et al.*, 1982, 1984; van den Broek *et al.*, 1986) and streptococcal cell wall (SCW) induced arthritis in rats (Cromartie *et al.*, 1977; Fox *et al.*, 1982; Wilder *et al.*, 1983). AIA is induced by injection of a highly cationic antigen into the knee joint of mice after immunization with the same antigen. Due to the electrostatic interaction of these cationic antigens with cartilage, the retention of antigen in the joint is high and the resulting arthritis is of a semi-chronic nature (van den Berg *et al.*, 1982, 1984). Besides antigen retention in the joint, a T cell response is obligatory for AIA in mice (Brackertz, Mitchell & Mackay, 1977; van Lent *et al.*, 1987). In this animal model, the subsided arthritis can be reactivated by systemic challenge with antigen. These so-called flares have been shown to be dependent on local hyperreactivity in the arthritic joints which is mediated by retained T cells (Lens, van den Berg & van de Putte, 1984a; Lens *et al.*, 1984b; van den Broek *et al.*, 1986), and might contribute to chronicity. If instead of a cationic protein antigen, SCW are injected intra-articularly in mice, very similar phenomena are observed (van den Broek *et al.*, 1988c). Injection of SCW into the joints of Lewis rats also induces a transient monoarthritis that can be reactivated by systemic challenge with homologous or heterologous cell walls or with bacterial lipopolysaccharide (LPS) (Esser *et al.*, 1985, 1986; Stimpson *et al.*, 1987).

When a high dose of SCW is injected intraperitoneally as a sterile, aqueous suspension, a persisting polyarthritis occurs in Lewis, but not in nude Lewis (Ridge *et al.*, 1985) or resistant F344 rats (Cromartie *et al.*, 1977). It has been shown that bacterial fragments disseminate to the joints where they persist for prolonged periods of time, but the bulk of the injected fragments are sequestered away from the joint, especially in the liver, spleen and bone marrow (Eisenberg *et al.*, 1982; Wilder *et al.*, 1983; Allen, Calandra & Wilder, 1983; Allen *et al.*, 1985; Stimpson *et al.*, 1986a). These extra-articular SCW depots may

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explain in part why chronic relapsing arthritis follows i.p. injection, whereas only transient inflammation is seen after local injection: from these depots small amounts of SCW may leak into the joint continuously, inducing a perpetuous flare of the arthritis and chronic disease.

Previous studies on this chronic SCW model showed a strong genetic influence and a T cell dependence with respect to the chronic phase. Despite ample investigations, no differences between the two strains could be detected in distribution and clearance of cell walls (Wilder *et al.*, 1983; Anderle *et al.*, 1985), cutaneous inflammatory reactions to SCW (Allen *et al.*, 1983) and SCW-specific antibody responses (Greenblatt, Hunter & Schwab, 1980). At present, the evidence for a crucial role for T cells in chronic SCW-induced arthritis (Ridge *et al.*, 1985; Yochum *et al.*, 1986; van den Broek *et al.*, 1988a) is growing and recent studies from our laboratory suggest an important role for SCW-specific T cells (van den Broek *et al.*, 1988a): Lewis rats mount anti-SCW T cell responses, while F344 rats are completely deficient in this. The unresponsiveness in F344 rats is partly controlled by suppressor T cells (van den Broek *et al.*, 1988a) and dominance of suppression may explain the lack of SCW-specific T cell responses and thus the lack of T cell dependent exacerbations and chronic arthritis in this strain.

To investigate our hypothesis that in the i.p. SCW-model chronicity is a continuous, T cell dependent flare, we studied the role of T cells in flare-up reactions and we subsequently compared the susceptibility of Lewis and F344 rats with these exacerbations of SCW-induced monoarthritis.

MATERIALS AND METHODS

Rats

Female Lewis rats were obtained from Zentral Institut für Versuchtierzucht (Hannover, FRG) and female F344 rats were obtained from Harlan Sprague Dawley (Indianapolis, IN). Both strains were housed in our animal laboratory, were fed a standard diet and tap water *ad libitum*.

Rats weighed 100–125 g at the start of the experiments.

Bacteria

The following bacterial strains were used: *Streptococcus pyogenes*, group A type 12; *Lactobacillus casei* (ATCC 11578); *S. faecium*, ATCC 9790, group D (gift of Dr G. D. Shockman, Temple University School of Medicine, Philadelphia, PA); *Propionibacterium acnes*, VPI 0009 and *Peptostreptococcus productus*, VPI C18-23 (both a gift of Dr C. S. Cummins, Virginia Polytechnic Institute Anaerobe Laboratory Culture Collection) and *Methanobacterium formicicum*, JF-1 (gift of Dr J. G. Ferry, Virginia Polytechnic Institute and State University).

Cell wall purification

Bacteria were grown to late log/early stationary phase in Todd Hewitt broth, harvested and disrupted by shaking with glass beads. The material was treated with DNAse (4 h, 37°C) and trypsine (4 h, 37°C), sonication and cell walls were isolated by differential centrifugation; fragments sedimenting at 100 000 ***g***, but not at 10 000 ***g***, were used. The purification and chemical analysis has been described in detail (Stimpson *et al.*, 1986a); all consist of complexes of peptidogylcan and polysaccharide, or pseudomurein and polysaccharide in the case of *M. formicicum*.

Arthritis induction by systemic administration of SCW

To induce a chronic polyarthritis, F344 and Lewis rats were injected with SCW (100 μg dry weight/g body weight) intraperitoneally. The arthritis was scored macroscopically, by measuring paw thickness with an industrial micrometer and by histology.

Induction of arthritis by intra-articular injection of SCW

Unilateral inflammation of the knee joint was induced by intra-articular injection of 25 μg dry weight SCW in 10 μl phosphate-buffered saline (PBS) into the right knee joint of F344 and Lewis rats. In some experiments right ankle joints (instead of knees) were injected with 5 μg SCW suspended in 10 μl PBS. The same volume of PBS was injected into the left joint as a control.

Induction of flare-up reaction

An exacerbation of the unilateral arthritis was induced in rats at several times after arthritis induction as indicated in results by i.v. injection of 300 μg (dry weight) homologous or heterologous cell walls in PBS.

Measurement of joint inflammation

Arthritis and flare up reaction were measured by the ^{99m}Tc uptake method (van den Berg *et al.*, 1984). Briefly, rats were anaesthesized with pentobarbital (300 mg/kg) and 0·2 ml (7·4 MBq/ml) ^{99m}Tc was administered subcutaneously. After 30 min the ^{99m}Tc uptake in the right and the left knee joint was measured by external gamma counting. The uptake of this small radioisotope is a measure for local blood flow and oedema. The severity of inflammation is expressed as right-over-left (R:L) ratios and all values exceeding 1·10 were assigned as inflammation. In the case of exacerbated ankle joints, the flare-up was clinically scored on a scale from 0–4 by an observer who was unaware of the treatment of the rats.

Joint inflammation was also scored histologically: knee joints were removed *in toto* and processed for histology as described (van den Berg *et al.*, 1982). Sections (7 μm) were stained with haematoxilin and eosin. Scoring of inflammation was done by two independent observers on coded slides.

Depletion of T cells

Monoclonal antibodies ER1 and ER3 are mouse IgG2a antibodies directed against the rat W3/13 or the rat OX8 antigen respectively (Joling *et al.*, 1985) and thus reactive with all T cells (ER1) or only with cytotoxic and suppressor T cells (ER3). Monoclonal antibodies ER1 and ER3 were kindly provided by Dr J. Rozing (Department of Immunology, TNO-IVEG, Rijswijk, the Netherlands). Monoclonal antibody WT32 is a mouse IgG2a directed against the human CD3 antigen (Tax *et al.*, 1984) and was kindly provided by Dr W. J. M. Tax (Department of Nephrology, University Hospital Nijmegen). WT32 was used as a control monoclonal antibody. ER1, ER3 and WT32 were cytotoxic to target cells in combination with rabbit complement and had a cytotoxic titre (dilution at which 50% of maximal lysis was observed) of 1:8000.

Monoclonal antibodies were administered to rats by i.p. injection of 1 ml of crude ascites once every 2 days, starting at the day of intra-articular injection of cell walls.

To check depletion of (suppressor) T cells, spleens were removed from ER1-, ER3- and WT32-treated rats. A single cell suspension was made and erythrocytes were lysed. The cells

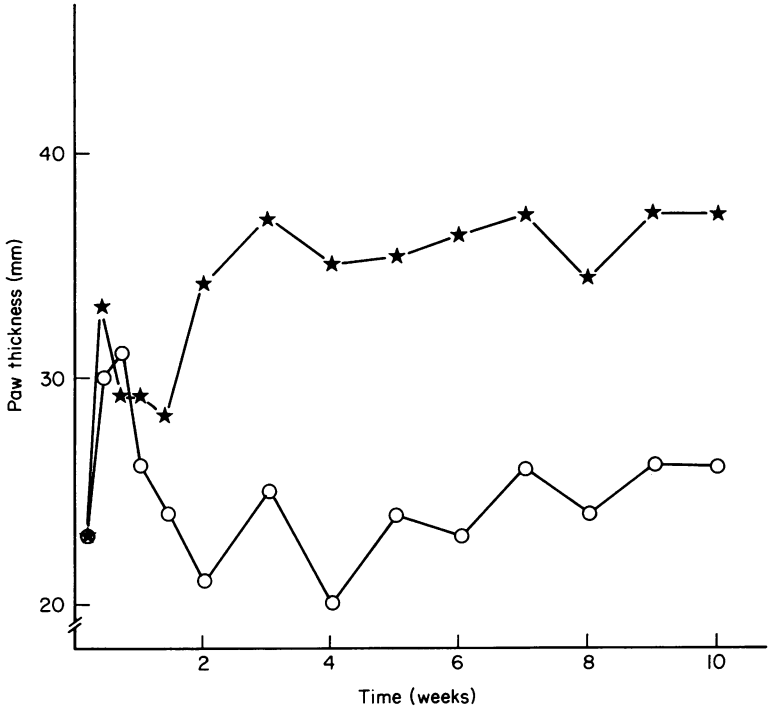

Fig. 1. Polyarthritis in F344 (○) and in Lewis (★) rats induced by one intraperitoneal injection of a sterile aqueous suspension of streptococcal cell walls (100 μg dry weight/g body weight). The arthritis is scored by measuring the sum of paw thickness with an industrial micrometer. The values are the mean from a group of five rats.

were adjusted to a concentration of 10^7/ml in ice-cold PBS+ 0·5% bovine serum albumin (BSA)+0·05% NaN_3 (IF buffer). All subsequent steps were carried out on ice; 100 μl cells were incubated with 100 μl of a 1:40 dilution of the following murine monoclonal antibodies: W3/25 (rat helper T cells), OX8 (rat cytotoxic/suppressor T cells) and W3/13 (rat *pan*-T cell) (all Seralab). For a blank value, cells were incubated with 100 μl IF buffer alone. After 30 min the cells were washed twice with IF buffer and incubated with 200 μl of 1:50 diluted fluorescein isothiocyanate (FITC) conjugated goat anti-mouse IgG (Nordic, Tilburg, the Netherlands) for 30 min. After three washes, the cells were suspended in 100 μl PBS and 100 μl 2% paraformaldehyde were added.

Percentages of positive cells were scored by two independent observers on coded slides.

Proliferative response of SCW-specific T cells to homologous and heterologous cell walls

To correlate the capacity of heterologous cells walls to induce a reactivation of SCW-induced arthritis with the ability of these walls to induce proliferation of SCW-primed T cells, we immunized female Lewis rats with SCW emulsified in Freund's incomplete Adjuvant (FIA). Each rat was injected with 100 μg SCW into both foot-pads of the fore-paws. After 9 days, the draining lymph nodes were removed aseptically and a single cell suspension was made. The cells were suspended at 10^6/ml in RPMI 1640 Dutch modification supplemented with 10 mM glutamin, 20 mM pyruvate, 40 μg/ml gentamycin (Flow Lab) and 2% (v/v) SF1 (Costar). One-hundred microlitres of this suspension were incubated in round-bottomed microtitre plates (Costar) with 100 μl of the following stimuli (final concentration): medium alone; concanavalin A (1 μg/ml); cell walls (30, 10, 3 and 1 μg/ml) from *S. pyogenes*, *S. faecium*, *P. productus*, *L. casei*, *P. acnes* and *M. formicicum*. After 3 days, $3·7 \times 10^4$ Bq (= 1 μCi) ^{3}H-thymidine (Amersham Nederland; specific activity $0·7–1·1 \times 10^8$ Bq/mmol) were added to each well. After 24 h, the cells were harvested onto glass fibre filters and the amount of incorporated radioactivity was counted in a liquid scintillation analyser. All values are expressed as the mean of at least triplicate cultures.

Statistical analysis

The R:L ratios of ^{99m}Tc uptake of different experimental groups were compared statistically using the two-tailed Mann–Whitney *U*-test.

RESULTS

Arthritis in F344 and Lewis rats after systemic administration of SCW

Figure 1 shows the arthritis measured as the sum of the increased paw thickness, induced by i.p. injection of 100 μg dry weight SCW/g body weight. This dose has been proven to be arthritogenic in female Lewis rats (van den Broek *et al.*, 1988a). Lewis rats developed an acute as well as a chronic joint inflammation after SCW injection, whereas F344 rats only developed the acute phase of the disease. These findings are in concordance with the results of others (Wilder *et al.*, 1982, 1983).

Arthritis in F344 and Lewis rats after intra-articular injection of SCW

In contrast to systemic administration, intra-articular injection of SCW leads to a transient arthritis (van den Broek *et al.*, 1988c;

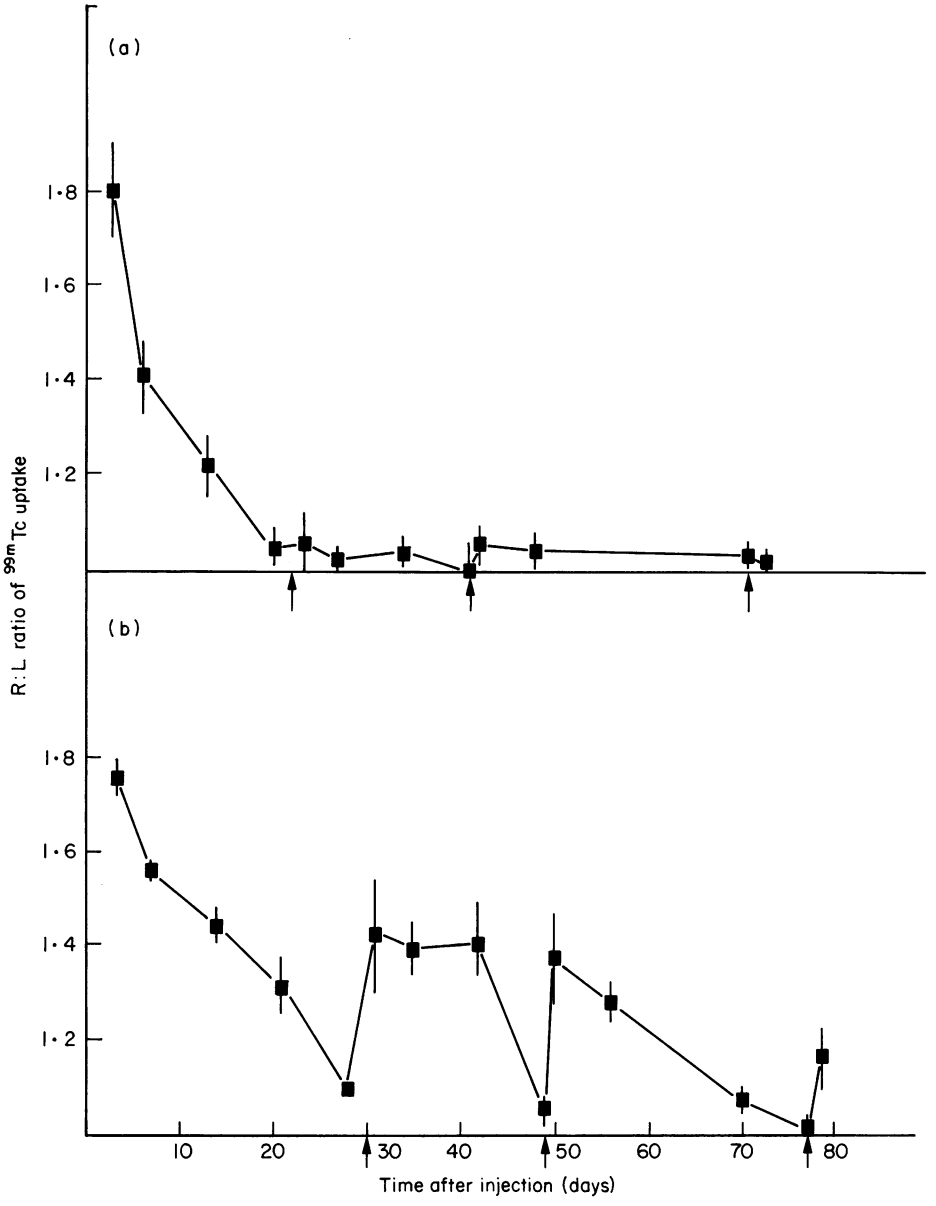

Fig. 2. Unilateral arthritis and flare-up reaction in F344 (a) and Lewis (b) rats. An acute course of arthritis is induced by intra-articular injection of 25 μg streptococcal cell wall (SCW). A flare-up reaction is induced by i.v. injection of 300 μg SCW at the times indicated by an arrow. The inflammation is scored by determining the ratio of ^{99m}Tc uptake in the right (arthritic) over the left (control) knee joint (R:L ratio). The values represent the mean ± s.d. from groups of six rats.

Esser *et al.*, 1985, 1986) in the injected joint only. The Lewis as well as the F344 rat strain develop a monoarthritis, which is comparable in severity during the first week (Fig. 2). However, the arthritis in Lewis rats was significantly more protracted than that in F344 rats. This tendency can already be observed at day 7, and the difference is statistically significant at all points thereafter.

Flare-up reactions of unilateral SCW-induced arthritis: comparison of Lewis and F344 rats

Previous studies from our laboratory have demonstrated that flare-up reactions of antigen-induced and SCW-induced arthritis in mice are dependent on helper T cells (Lens *et al.*, 1984b; van den Broek *et al.*, 1986). There is also ample evidence for T cell dependence of the chronic phase of arthritis induced by i.p. injection of SCW (Ridge *et al.*, 1985; Yochum *et al.*, 1986; Wilder, Allen & Hansen, 1987). The fact that F344 rats do not develop chronic joint inflammation (Wilder *et al.*, 1982, 1983) and that this strain mounts immunosuppression rather than help (van den Broek *et al.*, 1988a) upon injection with SCW, provided a rationale for studying flare-up reactions in Lewis and F344 rats.

The flare-up reaction induced by i.v. challenge with 300 μg SCW (one representative experiment out of four) is shown in Fig. 2. We induced a flare-up reaction no sooner than when the background R:L ratio was 1·10 or less. The R:L ratios of ^{99m}Tc uptake in F344 and Lewis rats are shown, resulting from repeated i.v. challenges with SCW. At least once before each

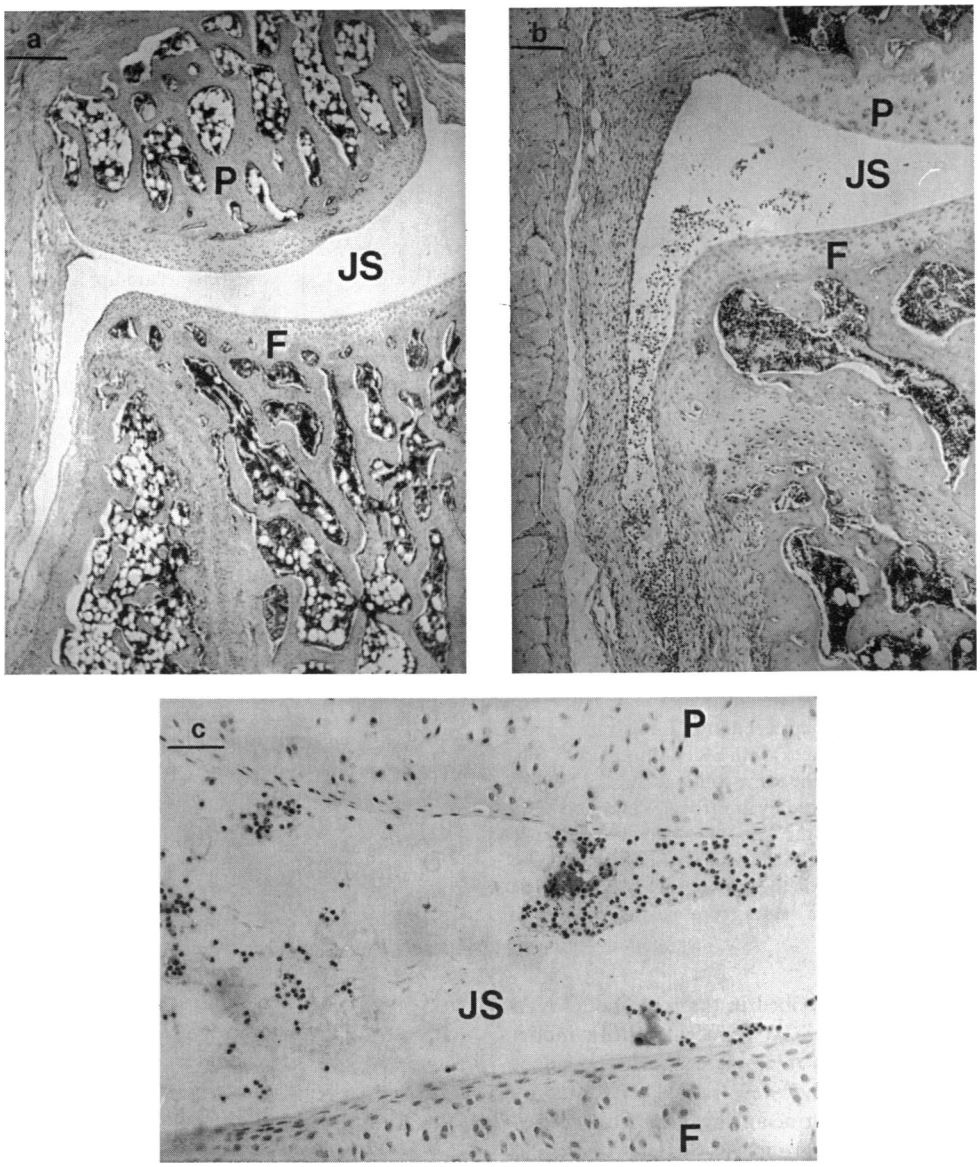

Fig. 3. Histology of flare-up reaction and background arthritis in rat knee joints. (a) Flare-up reaction, F344, magnification ×18; (b) flare-up reaction, Lewis, magnification ×73; and (c) as (b), magnification ×180. Haematoxylin and eosin staining. P, patella; JS, joint space; F, femur.

Table 1. Histological scores of flare-up reactions

Rat strain	Treatment	*n*	Infiltrate*	Exudate*	Granulocytes†
Lewis	None	15	1·0	2·1	2·2
Lewis	WT32	13	0·9	2·1	1·9
Lewis	ER1	7	0·3	0·4	0·4
F344	None	15	0·5	0·4	0·5
F344	WT32	15	0·4	0·3	0·6
F344	ER3	15	0·4	0·4	0·6

* Infiltrate and exudate are scored using a scale from 0 to 3 by two independent observers on coded, H&E stained sections. 0 = no infiltrate/exudate, 3 = large mass of infiltrate/exudate.

† Scored as a percentage of granulocytes present in inflammatory mass. 0 < 25%; 1, 25–50%; 2, 50–75%; 3 > 75%.

challenge the inflammation was measured, to ensure that the arthritis had waned to a sufficient extent. Despite several i.v. challenges, the F344 rat remained 'resistant' to exacerbations of the arthritis, while repeated flare-ups could be induced in the Lewis rat.

Figure 3 and Table 1 give a histological view on the flare-up reaction in Lewis and the absence of it in F344 rats: a flare-up reaction is characterized by an infiltrate, but more importantly by an exudate that consists mainly of granulocytes. These features are rarely seen in a waning arthritis (data not shown). After the peak of the reactivation, the amount of granulocytes decreases while the amount of mononuclear cells increases again.

Effect of T cell depletion on the flare-up reaction in Lewis rats

To investigate whether the flare-up reaction in this model—

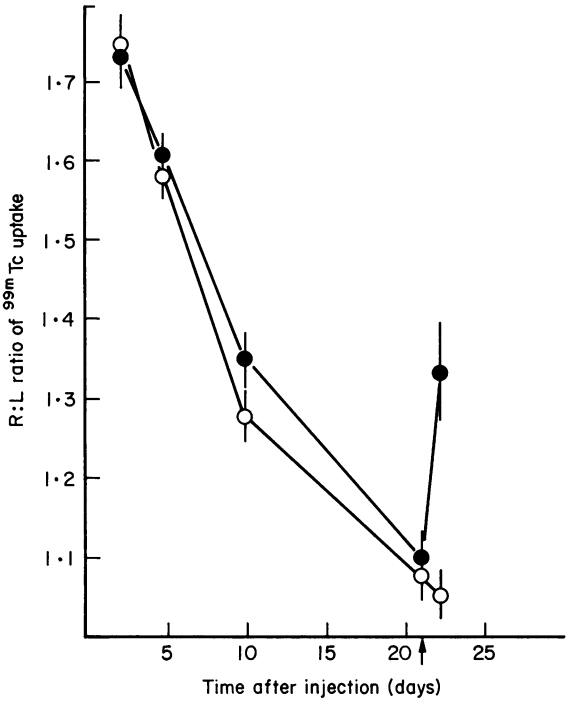

Fig. 4. Unilateral arthritis and flare-up reaction in Lewis rats treated from day 0 through 21 (three times a week) with ER1 (anti-rat *pan*-T cell, ○) or with a control monoclonal of the same subclass (IgG2a), WT32 (anti-human T3, ●). Arthritis is induced by intrarticular injection of 25 μg streptococcal cell wall (SCW) and the flare-up reaction is induced 21 days later by i.v. challenge with 300 μg SCW. The arthritis is scored by determining the ratio of ^{99m}Tc uptake in the right (arthritic) over the left (control) knee joint (R:L ratio). The values represent the mean ± s.d. from groups of six rats.

analogous to what has been described in the AIA model (van den Broek *et al.*, 1986) and the SCW-induced arthritis model (van den Broek *et al.*, 1988c) in mice—is a T cell dependent reaction, we depleted T lymphocytes from Lewis rats by administration of a cytotoxic murine anti-rat *pan*-T cell monoclonal antibody (ER1). A control group was treated with WT32, a monoclonal antibody against human CD3 of the same subclass (IgG2a). Three weeks after intra-articular administration of 25 μg SCW, rats were challenged intravenously with 300 μg SCW and 24 h later the ^{99m}Tc uptake was measured to assess the occurrence of the flare-up reaction.

Figure 4 shows the R:L ratios of ^{99m}Tc uptake at different times after intra-articular injection of SCW in ER1- and WT32-treated groups ($n=6$) and after i.v. challenge: in T cell depleted rats the arthritis could not be reactivated by i.v. challenge, while in the WT32-treated control group a distinct flare-up was measurable ($P<0{\cdot}005$). Immediately after measuring the flare-up reaction, rats were killed and the right and left knee joints were removed for histology and the spleen was removed to check T cell depletion by immunofluorescence. Histological analysis confirmed the presence of acute inflammation in the WT32-treated group and the absence of a reaction in the ER1-treated group. Immunofluorescence showed that in spleens of the WT32-treated group 45 ± 6% and in spleens of the ER1-treated group 3 ± 3% of the cells bound W3/13.

These data prove that SCW-induced flare-up reactions in rats are dependent on T lymphocytes.

Table 2. Correlation between the ability of heterologous cell walls to induce flare-up reactions of streptococcal cell wall (SCW)-induced unilateral arthritis *in vivo* and to stimulate SCW-specific T lymphocytes *in vitro*

Cell walls (10 μg/ml)	Severity of flare-up reaction*	Proliferative response as % of the response to the priming antigen† Experiment no. 1	2	3
Streptococcus pyogenes	Severe‡	100	100	100
S. faecium	Severe‡	63	64	69
Peptostreptococcus productus	Moderate‡	30	20	23
Lactobacillus casei	None§	9	7	3
Propionibacterium acnes	None‡	7	11	3
Methanobacterium formicicum	None‡	7	3	6

* On day 0 an acute course of arthritis is induced by intra-articular injection of 5 μg of SCW into ankle joints. A flare-up reaction is induced by i.v. injection of 300 μg cell walls from various bacteria 17–25 days after induction of arthritis.

† Rats were immunized with SCW/Freund's incomplete adjuvant, and 9 days later cells were isolated from the draining lymph nodes; 10^5 cells were incubated with 10 μg/ml of the various cell walls and the resulting proliferation was measured as described in Materials and Methods. Values represent the mean of quadruplicate wells. The proliferative response (stimulation index = counts due to stimulus/counts due to medium) induced by homologous cell walls is set at 100% and the responses induced by heterologous cell walls are relative to that value.

‡ Esser *et al.*, 1985.

§ Unpublished data.

Table 3. Proliferative responses to bacterial cell walls of naive lymph node or spleen cells and ovalbumin-primed lymph node cells *in vitro*

Stimulus* *in vitro*	Naive Lymph node	Naive Spleen	Ovalbumin-primed Lymph node
None	534	1621	925
Streptococcus pyogenes	611	1821	1193
S. faecium	614	1638	1036
Peptostreptococcus productus	651	1493	1094
Lactobacillus casei	602	1284	1004
Propionibacterium acnes	513	1650	1248
Methanobacterium formicicum	565	1583	1205
Concanavalin A	39983	60926	49367

For methods, see table 2; results are the mean of quadruplicate cultures and the s.d. was always within 10%. Values are expressed as ct/min.

* Cell walls had a final concentration of 30 μg/ml, and concanavalin A of 1·25 μg/ml.

Effect of OX8$^+$-cell depletion on the susceptibility for flare-up reactions in F344 rats

Since a recent study from our department (van den Broek *et al.*, 1988a) showed that depletion of the OX8$^+$ subset *in vitro* led to a partial restoration of the suppressed antigen- and mitogen-driven proliferative response in SCW-primed F344 rats, we investigated whether depletion of this subset *in vivo* rendered this strain susceptible to reactivations of SCW-induced arthritis. For this purpose, F344 rats were treated with ER3 and controls were treated with WT32, as indicated in Materials and Methods. Lewis rats served as positive controls. Only the Lewis rats are susceptible to exacerbations of the monoarthritis and both groups of F344 rats remain resistant (Table 1), despite an intact helper T cell population and the successful depletion of suppressor/cytotoxic T cells (spleens from WT32-treated F344 rats—OX8$^+$, 23$\pm$5%, W3/25$^+$, 39$\pm$6%; spleens from ER3-treated rats—OX8$^+$, 1$\pm$2%, W3/25$^+$, 36$\pm$6%). Therefore, we conclude that the OX8$^+$ subset is not the (only) regulatory cell population to determine the susceptibility for exacerbations and maybe even for chronic cell wall-induced arthritis.

Reactivation of SCW-induced arthritis in Lewis rats by homologous and heterologous cell walls and a correlation to T cell response

Since bacterial cell walls are structurally related to each other, an important question has been the potential of heterologous cell walls to reactivate a SCW-induced unilateral arthritis. Past studies (Esser *et al.*, 1985; Stimpson *et al.*, 1987) have established that an arthritis which is induced by one bacterium can be reactivated by certain, not necessarily related other bacteria, thus providing a plausible mechanism for maintenance of a joint inflammation.

Several systems, including this study, have demonstrated amply (Lens *et al.*, 1984b; van den Broek *et al.*, 1986, 1988c) that exacerbations of arthritis are dependent on antigen-specific T lymphocytes, and therefore we tried to correlate the capacity of cell walls from various bacteria to induce a flare-up of SCW-arthritis to the potential of these cell walls to stimulate SCW-specific T lymphocytes. The responses are expressed as a percentage from the 100% value induced by the homologous cell walls. Cell walls from bacteria that induced a maximal flare-up reaction (*S. pyogenes* and *S. faecium*) induced the highest proliferative response of SCW-primes T cells *in vitro* (Table 2), cell walls from *P. productus*, which induce a minor exacerbation, also induce a lower degree of proliferation, while cell walls from *P. acnes*, *L. casei* and *M. formicicum* (incapable of reactivation) induced no more than a slight response of SCW-primed cells. Table 3 shows that neither naive lymph node or spleen cells, nor ovalbumin-primed lymph node cells proliferate in response to cell walls *in vitro*, thus excluding the cell wall preparation being a T cell and/or B cell mitogen. These data directly support the crucial role of antigen-specific T lymphocytes in the induction of reactivations of joint inflammation.

DISCUSSION

This study was designed to shed some light on the pathogenetic mechanism of SCW-induced polyarthritis in rats (Cromartie *et al.*, 1977), an animal model that is widely used to study chronic joint inflammation. Rats with this disease display a biphasic polyarthritis. The acute phase (day 1–3 after i.p. injection of SCW) has been shown to be independent of T lymphocytes (Ridge *et al.*, 1985; Yochum *et al.*, 1986; Wilder *et al.*, 1987) and dependent on complement (Schwab *et al.*, 1982). The mechanism of the chronic arthritis (starting after day 9–14) is still a matter of debate. It has been convincingly demonstrated, however, that the chronic joint inflammation is a T cell dependent phenomenon (Ridge *et al.*, 1985; Yochum *et al.*, 1986; Wilder *et al.*, 1987). Chronic arthritis is inducible only in susceptible rat strains, of which the female Lewis rat is the most commonly used; histocompatible F344 rats are resistant to chronic polyarthritis (Wilder *et al.*, 1982, 1983).

The persistence of SCW in the joint has been attributed a great deal of importance (Cromartie *et al.*, 1977; Eisenberg *et al.*, 1982; Allen *et al.*, 1983; Wilder *et al.*, 1983; Anderle *et al.*, 1985; van den Broek *et al.*, 1988c). This persistence, however, can not be in itself sufficient for chronic arthritis, because the distribution of cell walls in Lewis and F344 rats is similar (Wilder *et al.*, 1983; Anderle *et al.*, 1985). In addition, arthritis induced by intra-articular injection of 5 to 10 μg SCW is transient (10–14 days) (Esser *et al.*, 1985, 1986; Stimpson *et al.*, 1987), while the amount of SCW estimated to be in a hind-joint 1 day after i.p. injection of 10 mg is less than 10 μg (van den Broek *et al.*, 1988c), yet does cause chronic arthritis.

It may be relevant for the elucidation of the pathogenetic mechanism of the chronic arthritis to investigate the difference with respect to chronicity between the systemic and the intra-articular model. As a possible explanation for this disparity, we hypothesize that locally injected cell walls are locally sequestered (e.g. ingestion by macrophages) and not accessible for interaction with T cells any more after a certain period, while a continuous flow of antigen can reach the joint from large extra-articular pools as a result of systemic injection. Renewed influx of SCW into the joint can induce T cell-mediated exacerbations of the waning arthritis. Due to locally retained antigen-specific T cells, the joint is in a hyper-reactive state, and very minute amounts of antigen are sufficient to reactivate the waning inflammation. The potential of such a mechanism has been extensively illustrated by former studies by us (Lens *et al.*, 1984a, b; van den Broek *et al.*, 1986, 1988c) and others (Esser *et al.*, 1985; Stimpson *et al.*, 1987). Flare-up reactions with SCW as an antigen could even lead to more severe joint inflammation compared with those induced with protein antigens, because in addition to *in vivo* persistence, two important characteristics of SCW—immunogenicity and nonspecific inflammatory capacities—can work synergistically.

Our results provide evidence for the existence of the above-mentioned pathway. In accordance with studies from others (Cromartie *et al.*, 1977; Fox *et al.*, 1982; Wilder *et al.*, 1982, 1983; Esser *et al.*, 1985, 1986; Stimpson *et al.*, 1987), our results confirm that only Lewis rats had chronic polyarthritis after i.p. injection of cell walls (Fig. 1), while both in Lewis and in F344 rats a monoarthritis could be induced after intra-articular injection of SCW (Fig. 2). Intravenous challenge with SCW evoked a clear exacerbation of the waning arthritis in Lewis rats, but failed to affect the F344 rat. This striking difference was seen either when a similar background arthritis or the same time lapse after intra-articular was chosen to determine the moment of i.v. challenge. Figure 3 also shows that even more frequent i.v. challenges with cell walls did not lead to exacerbations in the F344 rat, whereas the Lewis rat flare-up reaction was present after each challenge. These findings correlate with the suscepti-

bility of Lewis and resistance of F344 rats to chronic arthritis in the i.p. SCW injection model, and support our hypothesis that repeated influxes of antigen into the joint can induce repeated exacerbations, and that these, if they occur within a short period of time, look like a chronic inflammation. Past studies of the distribution of ^{125}I-SCW polymers in rats after i.p. injection revealed significant levels of SCW in the peripheral blood for at least 30 days (Stimpson *et al.*, 1986b). In more recent studies, immunoreactive SCW was detected in blood for at least 57 days, without significant decrease in levels from day 6, after i.p. injection (Stimpson *et al.*, unpublished data). These observations support the idea that some steady state level of SCW exists in the circulation, presumably derived from large systemic depots, for long periods of time after injection, leading to chronic reexposure of the joint to SCW.

Since not every individual is susceptible to arthritis, it is interesting to find out how this resistance to exacerbations (and thus to chronic joint inflammation) is controlled. Our previous studies in the AIA model showed that flare-up reactions of inflammation are initiated by helper T cells (Lens *et al.*, 1984a, b; van den Broek *et al.*, 1986, 1988c). A recent *in vitro* study from our laboratory (van den Broek *et al.*, 1988c) revealed that in Lewis rats SCW-specific T cell responses could be demonstrated at different times (10–90 days) after different ways of priming (in saline or oil) with various doses (0·1–10 mg dry weight) of SCW, as contrasted with the F344 rat, in which severe immunosuppression was the result. To prove that as in the AIA model, exacerbations of SCW-induced monoarthritis are dependent on T cells, we treated Lewis rats with anti-*pan*-T cell monoclonal antibodies (ER1) and tried to induce a flare-up subsequently. No reactivation was inducible in T cell-depleted rats, while it was obviously present in a control group (Fig. 4), suggesting in this model flare-up reactions are dependent on T cells.

Another piece of evidence supporting the role of SCW-specific T cells in reactivations is shown in Table 2. Previous studies have demonstrated that a SCW-induced monoarthritis can be reactivated by an i.v. challenge with cell walls from some, but not all bacteria other than streptococci (Esser *et al.*, 1985; van den Broek *et al.*, 1988c). This observation has important implications for chronicity: it means that challenge with different bacterial species can be sufficient for maintenance of arthritis. Here we correlate the capacity of different cell wall types to induce a reactivation of SCW-induced monoarthritis with the capacity to induce proliferation of SCW-specific T lymphocytes *in vitro*. We conclude that SCW-specific T cells have to be stimulated by the challenging cell walls to a certain extent (stimulation index higher than 10–15) for induction of a reactivation of SCW arthritis. Interestingly, past work has shown that the amount of bacterial cell walls reaching the joint after i.v. injection is also correlated with the severity of the exacerbation (Esser *et al.*, 1985); we speculate that a specific T cell mediated response could lead to local changes (i.e. vascular permeability increase) which might in turn result in greater deposition of cell walls in the joint, thus amplifying the reaction.

All these data provide substantial evidence for the crucial role of (SCW-specific) T lymphocytes in the initiation of the flare-up reaction of a waning SCW-induced arthritis and thus in maintenance of the inflammatory process. These data may also explain why F344 rats are resistant to chronic bacterium-induced arthritis. The mechanism underlying the T cell unresponsiveness to SCW in F344 rats has yet to be elucidated. Recent *in vitro* studies from our department (van den Broek *et al.*, 1988a) showed that depletion of OX8$^+$ cells (suppressor/cytotoxic T cells) partly overcame the immunosuppression in F344 rats and thus led to a partial restoration of the SCW-specific T cell response. Therefore, in the present study the role of this immunoregulatory subset in flare-up reactions and, indirectly, in chronic arthritis was investigated by depleting F344 rats *in vivo* from OX8$^+$ cells. Surprisingly, depletion of this regulatory subset did not make this strain susceptible to exacerbations. This finding implies a discrepancy between our *in vivo* and *in vitro* data. However, there are conflicting reports on the role of OX8$^+$ cells in other experimental inflammatory/autoimmune diseases: studies in which OX8$^+$ cell depletion has affected the onset, down-regulation or severity of disease (Swierkosz & Swanborg, 1975; de Heer *et al.*, 1986; Caspi, Kuwabana & Nussenblatt, 1988; Sun *et al.*, 1988), and in which no effect was seen (Sedgwick, 1988) have been described.

One explanation for the lack of effect of suppressor cell depletion on the susceptibility for exacerbations of the F344 rat may be that the measured SCW-specific T cell response in the F344 rat *in vitro* after removal of OX8$^+$ cells is still too weak to have any biological meaning; Table 2 indeed shows that a slight stimulation of T cells is not sufficient. In addition, a proliferative response is not the same as a biological effect. Another explanation may be that despite removal of one regulatory subset, other subsets are still present *in vivo* to down-regulate T cell-specific reactions, as in adjuvant arthritis, where a CD4$^+$ T cell clone A2c has been shown to be capable of down-regulating or preventing arthritis (Cohen *et al.*, 1983, 1985). A third option could be that, unless a SCW-specific T cell response is (locally) present in the F344 rat after OX8$^+$ cell depletion, other factors in the F344 'environment' determine the *in vivo* observed overall response; for instance, the level of prostaglandin E (Fontana *et al.*, 1982; Wilder *et al.*, 1983), a known inhibitor of T cell responses, is higher in F344 than in Lewis rats after injection of SCW.

A recent finding (Sternberg *et al.*, 1989) suggests an interesting, novel view on immunoregulation in the SCW-induced arthritis: the activation of the hypothalamic–pituitary–adrenal axis by inflammatory stimuli (SCW, interleukin-1) was shown to be defective in SCW-arthritis susceptible Lewis rats, thus resulting in an incompetent feed-back loop; at the same time it was shown that resistant F344 rats could be made susceptible by treatment with a serotonin antagonist. These features might precede or exist besides the T cell unresponsiveness observed in F344 rats (van den Broek *et al.*, 1988a).

Taken together, we would suggest the involvement of T cells in chronicity of bacterium-induced polyarthritis on several points. Firstly, SCW-specific T cell responses develop in susceptible rat strains after priming (injection or infection). If, from a depot in the body or from another infection, small amounts of antigen perpetuously leak into the joints, repeated T cell-dependent exacerbations occur which then result in chronic arthritis. This explains the absolute dependence of the systemic model on T cells and suggests a way in which these SCW-specific T cells contribute to the maintenance of arthritis at the same time.

Secondly, the same SCW-specific T cells may be responsible

for the localization of the inflammation in the joints, while bacteria are present throughout the body. This is suggested by the finding (van Eden *et al.*, 1985; van den Broek *et al.*, 1988b) that bacteria can induce cross-reactive anti-cartilage responses in mice and rats. For maintenance of the arthritis, however, a continuous triggering by bacterial products seems to be obligatory (unpublished data).

The threat of a resulting autoimmune response may be the reason why such anti-bacterium responses are under stringent regulation in 'normal' individuals (e.g. F344 rats), thus resulting in a resistance to arthritis, while in 'arthritis-prone' individuals (e.g. Lewis rats) the regulatory equilibrium is easily disturbed and chronic arthritis can result from (continuous) bacterial priming. Host factors determining the susceptibility to SCW-induced arthritis are currently subjected to further study at our laboratory.

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