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Successful transfer of collagen-induced arthritis to severe combined immunodeficient (SCID) mice

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SUMMARY

We describe the adoptive transfer of erosive arthritis to an immunodeficient host. Spleen cells from arthritic DBA/1 mice (H-2q), immunized 4–6 weeks previously with bovine type II collagen in adjuvant, were transferred intraperitoneally into SCID mice (H-2d). SCID recipient mice also received native or denatured type II collagen (100 μg intraperitoneally) at the time of cell transfer. Arthritis developed in five out of five mice approximately 2 weeks after injection of cells plus native collagen, whereas animals injected with cells plus denatured collagen did not show any clinical or histological evidence of arthritis. The minimum graft size required for successful transfer of arthritis was established at 10^7 DBA/1 spleen cells. Histological examination of the joints of arthritic SCID recipient mice revealed synovitis, fibrosis and erosion of cartilage and underlying bone. Mean circulating levels of anti-type II collagen IgG were found to be significantly higher in mice injected with native collagen than those injected with denatured collagen (40 μg/ml and <1 μg/ml, respectively). The ability to transfer collagen-induced arthritis adoptively should facilitate the study of the cellular requirement and pathological mechanisms involved in the induction of this arthropathy.

Keywords collagen-induced arthritis adoptive transfer spleen cells SCID mice

INTRODUCTION

Immunization of genetically susceptible mice with type II collagen in adjuvant induces a polyarthritis which shares many features with human rheumatoid arthritis (RA). Both diseases demonstrate similar patterns of synovial infiltration and erosion of cartilage and bone, often in association with the formation of pannus. Increased MHC class II expression on cells in the synovium [1,2] and the presence of B and T lymphocytes specific for type II collagen have been demonstrated in collagen-induced arthritis [3,4] and in RA [5,6]. For these reasons, collagen-induced arthritis is considered to be a useful model for the study of the inflammatory processes common to both diseases.

Previous studies have reported transfer of arthritis by injection of serum from arthritic mice [7], or grafting of type II collagen-specific T cell clones [8]. However, only mild and transient pathological changes have been observed in the naive recipients, indicating that although T and B cells are both involved in the disease process, neither of these lymphocyte subsets alone is capable of causing severe, destructive arthritis.

Here we describe transfer of erosive arthritis by intraperitoneal injection of spleen cells from arthritic DBA/1 mice (H-2q) to SCID mice (H-2d). Mice of the SCID strain were chosen as recipients because they lack immunocompetent B and T cells [9], allowing the precise study of the lymphoid populations involved in pathogenesis, without interference by lymphocytes from the recipients. Furthermore, due to their immunodeficiency, SCID mice are unable to reject allografts and can therefore be used as 'universal' recipients.

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MATERIALS AND METHODS

Mice

DBA/1 mice were bred at the Kennedy Institute of Rheumatology and used at 8–12 weeks of age. Breeding pairs of C.B-17 *scid/scid* mice were obtained from the National Institute for Medical Research, London, UK. They were maintained under germ-free conditions using a specially adapted laminar flow cabinet and filter-top cages. Transfer experiments were carried out on mice aged about 8 weeks.

Type II collagen

Bovine articular cartilage was used as a source of type II collagen. Purification was by pepsin digestion and salt fractionation as described by Miller [10]. Denaturation of collagen was achieved by heating a solution of type II collagen (1 mg/ml) in Tris-buffered saline (TBS) to 60°C for 30 min.

Induction of arthritis in DBA/1 mice

Male mice were injected intradermally around the base of the tail with 200 μg of type II collagen dissolved in 0·1 M acetic acid and emulsified in Freund's complete adjuvant (FCA) (Difco Laboratories, East Molsey, UK). The incidence of arthritis was 70–90% and was macroscopically visible 4–6 weeks after immunization.

Transfer of arthritis

Spleens were removed aseptically from DBA/1 mice immediately after the onset of clinical arthritis. Single-cell suspensions were made by teasing apart the spleens in HBSS and pushing them through a metal sieve with a syringe barrel. Erythrocytes were lysed with ammonium chloride (0·83% w/v in Tris buffer), and the remaining cells were washed in HBSS, then injected intraperitoneally into male and female SCID recipients. Where indicated, mice also received an i.p. injection of either native or heat-denatured bovine type II collagen (100 μg, intraperitoneally) dissolved in TBS. The severity of arthritis was assessed in donor and recipient mice using a clinical scoring system of 0–2: 0, normal; 1, slight swelling and/or oedema; and 2, pronounced oedematous swelling. Each limb was graded in this way, giving a maximum score of 8 per mouse.

Histology

Feet were removed at autopsy and fixed in 10% buffered formalin. Following decalcification in buffered formalin, containing EDTA (5·5% w/v), the feet were embedded in paraffin, sectioned and stained with haemotoxylin and eosin.

Measurement of total IgG and collagen-specific IgG

Total murine IgG was measured by competition ELISA as previously described [11]. Sera were diluted in PBS containing Tween 20 (0·05% v/v), and mixed with goat anti-mouse IgG alkaline phosphatase conjugate (Sigma, Poole, UK). The serum/conjugate mixtures were incubated for 1 h at 37°C in microtitre plates previously blocked with casein in PBS (2% w/v), then transferred onto plates that had been coated with mouse IgG (10 μg/ml; Sigma). After 1 h at 37°C the plates were washed with PBS–Tween, then substrate (*p*-dinitrophenyl phosphate; Sigma) was added and allowed to develop for 1 h. Quantification of IgG was by reference to a standard curve of known IgG concentration.

Estimation of anti-type II collagen IgG levels was by direct ELISA. Microtitre plates were coated with bovine type II collagen (50 μl/well) in TBS (5 μg/ml). The plates were blocked for 1 h at room temperature with casein in PBS (2% w/v), then incubated with test sera serially diluted in PBS–Tween. After washing with PBS–Tween, the plates were incubated with goat anti-mouse IgG alkaline phosphatase conjugate (1:1000; Sigma). All incubations were carried out for 2 h at room temperature. After washing, the substrate (*p*-dinitrophenyl phosphate; Sigma) was added to the plates and allowed to develop for 1 h at 37°C. Optical densities were measured on a microplate reader (Biotec Instruments, Winooski, VT) at 405 nm. The concentrations of anti-collagen IgG contained in the test sera were determined by reference to a standard curve obtained by titrating a serum sample of known anti-collagen IgG concentration in parallel to the test sera.

Table 1. Transfer of arthritis requires cells from arthritic mice plus collagen II (1×10^7 spleen cells were injected with or without 100 μg type II collagen)

Donor immunization	Cells/collagen injected	Arthritis in recipients
Collagen/FCA*	Cells + collagen	4/6
Collagen/FCA*	Cells alone	0/6
FCA† alone	Cells + collagen	0/6

* Arthritic.
† Non-arthritic.

Table 2. Arthritis is transferred after concurrent administration of native type II collagen, but not denatured type II collagen (1×10^7 cells from arthritic donors were injected; 100 μg of native or heat denatured collagen were injected on the same day)

Cells/ collagen transferred	Cells + native collagen	Cells + denatured collagen	Cells alone
Arthritis in recipients	5/5	0/5	0/5
Anti-collagen IgG (μg/ml)	40 ± 13	< 1	< 1
Total IgG (μg/ml)	709 ± 184	713 ± 367	653 ± 302

Mean ± s.d.

Flow cytometry

Peritoneal exudate cells (PEC) were collected from SCID mice *post mortem* and analysed for size and granularity on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA).

RESULTS

Transfer of arthritis requires cells from collagen-immunized mice plus native collagen

Spleen cells from arthritic DBA/1 mice were injected into three groups each of six SCID mice. Mice from group 1 were given cells plus type II collagen (100 μg). Group 2 received cells alone, and group 3 received type II collagen plus cells from non-arthritic DBA/1 mice immunized with adjuvant alone. Arthritis developed in four out of six mice in group 1. None of the animals in groups 2 or 3 showed any clinical signs of disease (Table 1).

In order to ascertain whether it was necessary for the concurrently administered type II collagen to be in the native form in order for arthritis to develop in recipients, mice were given spleen cells from arthritic DBA/1 mice, plus 100 μg of either native or heat-denatured type II collagen. Arthritis was only transferred when native collagen was administered to the recipients of the spleen cells (Table 2).

The possibility of inducing arthritis in recipients by mitogenic rather than antigenic stimulation of donor lymphoid cells was investigated by injecting the recipients with spleen cells from

Table 3. Association between numbers of cells injected, onset of arthritis and level of anti-collagen IgG. All mice received spleen cells from arthritic DBA/1 mice and 100 μg collagen II

No. of cells injected	Arthritis in recipients	Day of onset (mean ± s.d.)	Anti-collagen IgG (μg/ml; mean ± s.d.)
1×10^6	0/3	—	6 ± 2
1×10^7	2/3	14·5 ± 2·1	86 ± 41
1×10^8	3/3	11·3 ± 1·1	276 ± 13

arthritic DBA/1 mice plus concanavalin A (Con A; 10 μg, intraperitoneally). Six days after injection, a mild erythema without swelling developed in all four treated mice. The affected feet returned to normal after 2–3 days (data not shown).

Antibody levels in donor and recipient mice

Circulating levels of total IgG and collagen-specific IgG were measured in recipient SCID mice simultaneously injected either with native (group 1), denatured (group 2) type II collagen, or saline (group 3). Mean (± s.d.) total IgG levels were similar in all three groups of mice (709 ± 184, 713 ± 367 and 653 ± 302 μg/ml, respectively). In contrast, circulating levels of specific anti-collagen IgG were very significantly higher in SCID mice receiving native collagen than in mice injected with either denatured collagen or no collagen at all (40 ± 13 μg/ml, < 1 μg/ml, and < 1 μg/ml, respectively; Table 2).

Number of cells required to transfer arthritis

In order to determine the minimum number of cells required to transfer arthritis, mice were given native type II collagen plus 10^6, 10^7, or 10^8 spleen cells from arthritic DBA/1 mice. All three mice grafted with 10^8 cells, and two out of three mice receiving 10^7 cells developed arthritis, whereas none of the animals transferred with 10^6 cells showed any signs of arthritis (Table 3). This demonstrates that the critical number of cells required to transfer arthritis consistently is between 10^7 and 10^8 spleen cells. In addition, there was a positive correlation between the number of cells injected and the level of anti-collagen IgG ($r = 0{\cdot}9$, $P < 0{\cdot}001$; see Table 3).

Comparison of arthritis in donor and recipient mice

The median clinical score in 14 arthritic SCID mice (pooled from the three experiments described above) was 6·0 (range 4·0–8·0). This was significantly higher ($P = 0{\cdot}003$) than the clinical score of 13 DBA/1 donors (median 3·0; range 2·0–8·0). Thus, arthritis was clinically more severe in the immunodeficient recipients than in the immunocompetent donors. In view of the severity of arthritis, mice were killed on the day when clinical disease became apparent, preventing subsequent studies of the long-term evolution of arthritis.

Microscopically, it was possible to detect severe inflammatory changes and marginal bone and cartilage erosion in arthritic SCID recipient mice (Fig. 1). The appearance of the inflamed synovia was similar in arthritic SCID recipient mice and DBA/1 donor mice, with neutrophils, fibroblasts and mononuclear cells dominating the inflammatory infiltrates.

Flow cytometry

The size and granularity of PEC from two arthritic SCID mice that had received cells and native collagen intraperitoneally were compared with PEC from two non-arthritic SCID mice that had received cells plus denatured collagen. Lymphocytes were defined and gated according to their characteristic forward light scatter and side light scatter patterns. Analysis of PEC from non-arthritic mice revealed a significant lymphocyte population. In contrast, minimal signal was detected in the lymphocyte gate when PEC from arthritic SCID mice were analysed (Fig. 2). This suggests an association between development of arthritis and lymphocyte migration away from the site of administration.

DISCUSSION

After immunization of DBA/1 mice with type II collagen, a number of immunological events are believed to occur which culminate, ultimately, in clinical arthritis. One method of studying these events is to evaluate changes in the expression of disease subsequent to the administration of immunomodulatory agents, such as cytotoxic or neutralizing antibodies. The major limitation of this approach is that it does not provide definitive data on specific aspects of the pathogenic process since this process involves a complex series of interrelated immunological, hormonal and even environmental factors. However, the ability to transfer arthritis consistently under controlled conditions and using a defined cell population provides the opportunity to study the immunological events associated with the induction of disease. Unfortunately, adoptive transfer of arthritis has been difficult to achieve [12]. Synovitis has been reported in nine out of 32 rats injected with lymphoid cells from syngeneic collagen-immunised rats [13] and injection of collagen-specific T cell lines, activated *in vitro* by antigen or Con A, has been shown to induce some transient inflammatory changes in the joints of naive hosts, although these were usually sub-clinical [8]. On the other hand, passive transfer of arthritis has been achieved by the injection of anti-collagen antibody-containing serum, either alone [7] or in conjunction with type II collagen-primed lymphoid cells [12,14].

Burrai *et al.* [15] transferred lymphocytes from rats with collagen-arthritis to naive, syngeneic recipients and found that arthritis could not be transferred by cells from arthritic or pre-arthritic donors, and that transfer of cells from collagen-immunized rats protected recipients from arthritis upon subsequent challenge with collagen in FCA. The ability of collagen-primed lymphoid cells to protect recipients from arthritis, as well as their relative inability to transfer disease, has been reported by other investigators in rat and mouse models of collagen-induced arthritis [16,17].

In the present study, severe destructive arthritis was transferred to immunodeficient mice using spleen cells from arthritic DBA/1 donor mice. 'Full-blown' arthritis only developed when type II collagen was administered at the time of transfer. When Con A was given in place of collagen a mild transient arthritis developed which may have been similar to the arthritis described by Holmdahl *et al.* [8] in DBA/1 mice that had been grafted with Con A-stimulated T cell lines.

It is clear from this study that there is a very strong association between the production of anti-type II collagen antibodies and the development of arthritis, suggesting a role

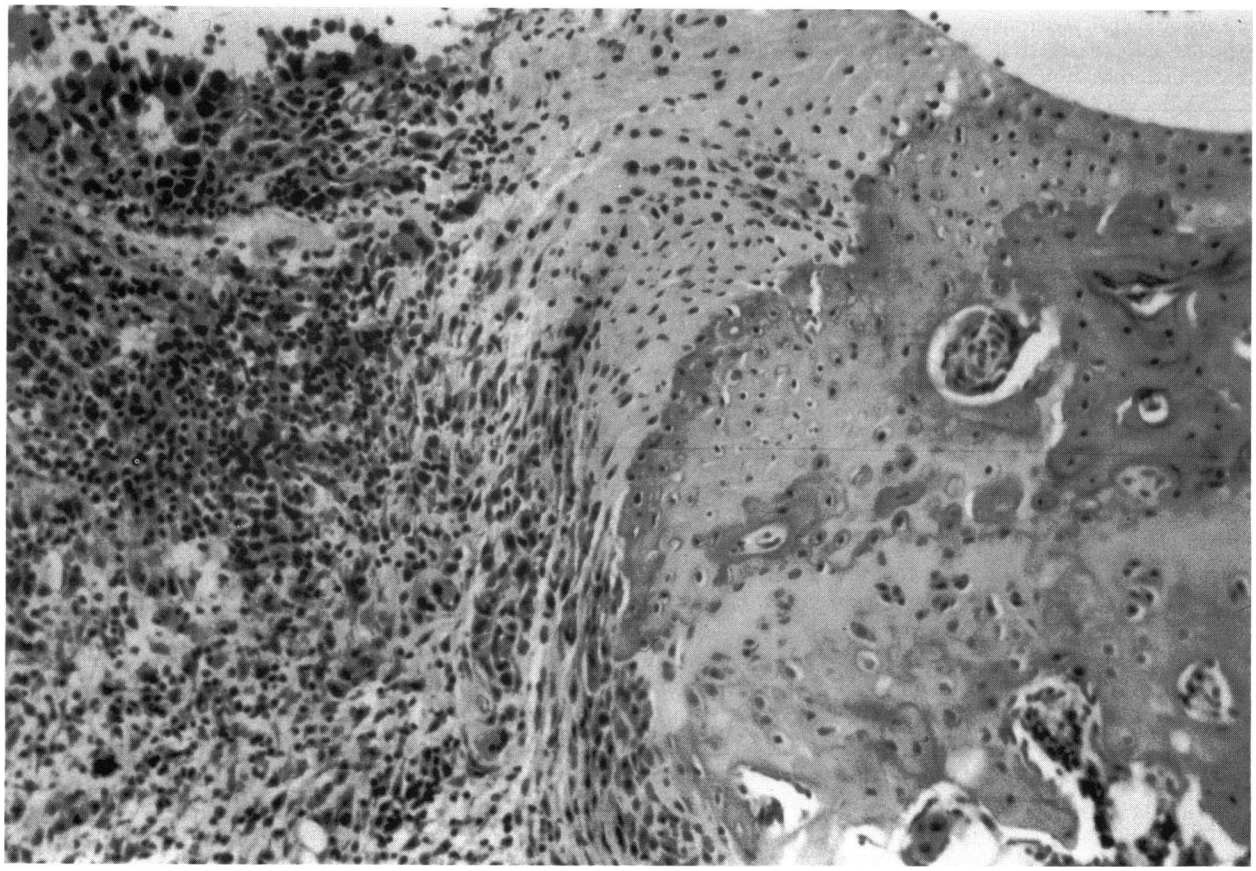

Fig. 1. Marginal erosion of cartilage and bone in arthritic SCID mouse 14 days after transfer of DBA/1 10^7 spleen cells and native type II collagen. Haematoxylin and eosin stain. Original magnification × 40.

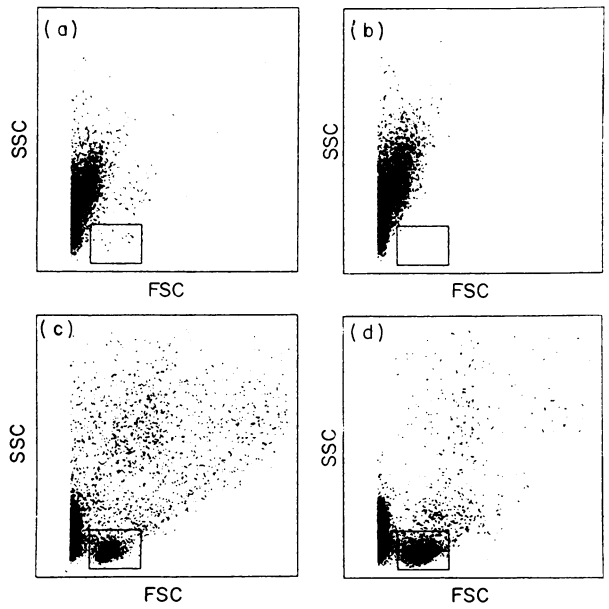

Fig. 2. Dual-parameter dot-plot of the forward (FSC) and side (SSC) light scatter of peritoneal cells from SCID mice 16 days after i.p. injection of 10^7 DBA/1 spleen cells. (a) and (b), cells from two arthritic mice given cells plus native type II collagen, (c) and (d) cells from two non-arthritic mice given cells plus denatured collagen. The area of lymphocyte cluster is shown by the box.

for such antibodies in the disease process. Anti-collagen IgG was always detected in the sera of animals which developed arthritis and negligible levels of this antibody were detected in non-arthritic recipient mice.

Bovine type II collagen was used to immunize the DBA/1 donor mice and arthritis developed in recipient mice when this same collagen was injected at the time of cell transfer. This demonstrates that antigen is required to activate (or reactivate) one or more splenocyte sub-populations in order for arthritis to develop. Presumably, autologous type II collagen, present in the cartilagenous tissues of the host, did not supply the necessary antigenic stimulus to the transferred cells. This suggests one of two possibilities. Firstly, murine cartilagenous collagen may be inaccessible to donor-derived cells. Thus, it may be necessary for soluble antigen to be brought into contact with these cells in the peritoneum before activation and migration towards the effector site can occur. Indeed, we have demonstrated conclusively that one of the features associated with the development of arthritis is the migration of lymphocytes out of the peritoneal cavity (Fig. 2). Another possibility is that transferred arthritogenic lymphoid cells may recognize epitopes present on bovine but not murine collagen, as has been suggested by Andersson & Holmdahl [18]. This could be tested by injecting recipient mice with murine type II collagen plus cells from bovine collagen-immunized donors.

Only native collagen was effective in inducing an arthritogenic response in the donated spleen cell population, indicating the possible presence of pathogenic T cell epitopes on native but not

denatured type II collagen. Alternatively, the critical activation step determining pathogenicity may be B cell rather than T cell-mediated since B cells, unlike T cells, are thought to react predominantly with conformation-dependent epitopes. The role of the B cell in this model may not be confined to the production of specific antibodies. These cells may produce cytokines critical to the development of arthritis, or may be involved in the presentation of antigen to T cells. There is evidence to suggest that the type of accessory cell that presents antigen to T cells determines whether an arthritogenic response ensues [19]. It should be possible to determine whether the role of the B cell in this model is confined to the production of antibodies by depleting the cell population used to transfer arthritis of B cells whilst administering anti-collagen antibodies, passively, to recipient mice.

Arthritis was clinically more severe in the immunodeficient recipients than in the immunocompetent donors. The reasons for this are not readily apparent, but several possible explanations exist. Firstly, the spleen cell population used to transfer arthritis may lack functional suppressor cells or transferred suppressor cells may be relatively short lived. Rudolphi *et al.* [20] have demonstrated that murine $CD4^+$ cells outlive $CD8^+$ cells after transfer into SCID mice. Secondly, it is possible that a suppressor network exists within the donor mice which serves to limit the severity of disease but which is absent from SCID mice. Activation of the suppressor arm of the immune response has been suggested as an explanation for the resistance to arthritis shown by recipients of collagen-primed T cells [17].

Chronic arthritis, attributable to graft-*versus*-host disease (GVHD), has been described in (BALB/c × A)F1 mice injected with histo-incompatible BALB/c spleen cells [21]. It is unlikely, however, that arthritis transferred from DBA/1 to SCID mice is caused by GVHD because of the requirement for native type II collagen and the fact that arthritis is transferrable only from collagen-immunized DBA/1 mice. Furthermore, in SCID mice reconstituted with histo-incompatible murine lymphocytes, GVHD is a phenomenon associated only with 'leaky' mice (mice with functional B and T cells) [22]. In our experiments, young SCID mice were used before the onset of leakiness.

The principal features associated with successful transfer of arthritis into SCID mice are the requirement for native type II collagen; the production of anti-type II collagen antibody; and the migration of lymphocytes away from the site of administration. Based on these findings, we are currently testing the hypothesis that after transfer to the SCID peritoneum, splenocytes from bovine type II collagen-immunized mice encounter soluble bovine type II collagen and are stimulated to synthesize lymphokines and anti-collagen antibodies and to upregulate the expression of cell surface adhesion molecules. The presence of circulating anti-bovine collagen antibodies which cross-react with murine collagen leads to the formation of immune complexes in the joint, resulting in activation of the complement cascade, and subsequently infiltration of inflammatory cells and the clinical manifestation of arthritis.

We have described a unique model of arthritis in which defined populations of arthritogenic cells can be studied *in vivo*. The absence of functional B and T cells in SCID mice prevents interference by host lymphocytes in the disease process and the histo-incompatibility of the two strains of mice makes antigen presentation by host to donor-derived T cells improbable. Selective depletion of the cell population used to transfer disease should allow the identification of cells involved in the induction of arthritis and the use of blocking antibodies should facilitate the study of the interactions between participating cells.

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