T and B Cell Recovery in Arthritis Adoptively Transferred to SCID Mice: Antigen-Specific Activation Is Required for Restoration of Autopathogenic CD4+ Th1 Cells in a Syngeneic System

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T cell homeostasis is a physiological function of the immune system that maintains a balance in the numbers and ratios of T cells at the periphery. A self-MHC/self-peptide ligand can induce weak (covert) signals via the TCR, thus providing an extended lifespan for naive T cells. A similar mechanism is responsible for the restoration of immune homeostasis in severe lymphopenic conditions such as those following irradiation or chemotherapy, or upon transfer of lymphocytes to Nuu/nu or SCID mice. To date, the genetic backgrounds of donor and recipient SCID mice were unmatched in all autoimmune arthritis transfer experiments, and the recovery of lymphoid cells in the host has not been followed. In this study, we present the adoptive transfer of proteoglycan (PG)-induced arthritis using unseparated and T or B cell-depleted lymphocytes from arthritic BALB/c donors to genetically matched syngeneic SCID recipient mice. We demonstrate that selectively recovered lymphoid subsets determine the clinical and immunological status of the recipient. We found that when T cells were depleted (>98% depleted), B cells did not produce PG-specific anti-mouse (auto) Abs unless SCID mice received a second Ag (PG) injection, which promoted the recovery of Ag-specific CD4+ Th cells. Reciprocally, as a result of B cell recovery, high levels of serum anti-PG Abs were found in SCID mice that received B cell-depleted (>99% depleted) T lymphocytes. Our results indicate a selective and highly effective cooperation between CD4+ T cells and B lymphocytes that is required for the restoration of pathological homeostasis and development of autoimmune arthritis in SCID mice. The Journal of Immunology, 2002, 168: 6013–6021.

Proteoglycan (aggrecan)-induced arthritis (PGIA)3 is a murine model of rheumatoid arthritis as indicated by clinical assessment, laboratory tests, and histopathology of the peripheral joints (1, 2). Arthritis can be induced only in genetically susceptible BALB/c or C3H mice by systemic immunization with human cartilage proteoglycan (PG; Refs. 1–4). The development of the disease is based upon cross-reactive immune responses between the immunizing human and mouse (self) cartilage PGs (3–5).

Several lines of evidence indicate that CD4+ Th cells play an important role in PGIA. 1) Susceptibility to PGIA is influenced by MHC (H-2d haplotype in BALB/c and H-2k in C3H mice) (3, 6–9); 2) immunization of BALB/c mice with PG induces a Th1-dominant T cell response (10, 11); 3) PG-specific Th1-type T cell hybridoma induces arthritis in BALB/c mice (12); 4) the disease is prevented when CD4+ T cells are depleted either in vitro (13) or in vivo (14); and, lastly, 5) treatment of arthritic mice with IL-4 can prevent disease development by inducing a switch from a Th1-type to a Th2-type response (10). Furthermore, IL-4-deficient mice in BALB/c background develop a significantly more severe disease when compared with wild-type BALB/c mice, and show a skewed Th1/Th2 profile with Th1 dominance (10, 11, 15, 16). Although the critical function of CD4+ T cells was implicated in arthritis induction (16), the role of Abs to mouse (self) PG (mPG) and/or B cells in the pathogenesis of this autoimmune model is not yet fully understood (5, 6, 13). Transfer of the disease required both T and B cells, and neither anti-PG Abs nor PG-specific B cells alone were able to transfer disease (13, 17). In contrast, PG-specific B cells appeared to play a major role in Ag presentation (5). Together, a highly specific cooperation between Ag-primed CD4+ Th1 and B cells appears to be critical for the development of the disease (6, 11, 16). T cell homeostasis is a physiological function of the immune system that maintains a balance in the numbers and ratios of T cells at the periphery (18–20). For long-term survival of naive T cells, a weak interaction between the TCR and a self-MHC/(self)peptide ligand is necessary (20, 21). Such “covert” signals provide T cells with an extended lifespan, and capacity to proliferate and populate the lymphoid organs (18, 19, 22). Homeostatic polyclonal T cell proliferation, i.e., the restoration of the original T cell pool, occurs in severe lymphopenic conditions such as those following irradiation, chemotherapy, or upon transfer of lymphocytes to Nuu/nu or SCID mice. A low number of T cells can repopulate the lymphoid organs, but only in syngeneic condition which allows for recognition of a wide range of self-MHC-associated/(self)peptide ligands by matched TCRs (23–25). Thus, the adoptive transfer of PGIA from BALB/c into genetically matched (both MHC and non-MHC genes) SCID mice seems to be an ideal model.

3 Abbreviations used in this paper: PGIA, proteoglycan-induced arthritis; PG, proteoglycan; CIA, collagen-induced arthritis; hPG, human PG; mPG, mouse PG; SI, stimulation index.
system for studying T cell restoration in a T cell-mediated autoimmune disease.

Human and experimentally induced autoimmune diseases exhibit complex and polygenic modes of inheritance, dictated by both MHC and non-MHC genes (7, 8, 26–30). In previous studies, collagen-induced arthritis (CIA) was transferred to genetically unmatched SCID mice (31–33). However, disparate genetic backgrounds might significantly modulate the cell survival and the outcome of immune reactions, even in an immune-compromised host. Furthermore, anti-collagen Abs play critical roles in CIA (either in the primary or transferred form of the disease; Refs. 34, 35), whereas PG-specific Abs appear to have only a limited function in arthritis induction in PGIA (6, 13). In this study, we present adoptive transfer of autoimmune polyarthritis in a syngeneic system to SCID mice. We focus on the recovery and clonal restoration of T and B cells, and demonstrate the role of these selectively recovered lymphoid cells in the regulation of the host’s (recipient) clinical and immunological status.

Materials and Methods

Ags, animals, and immunization

High-density cartilage PG (aggrecan) was purified from human articular cartilage by CsCl gradient centrifugation, and depleted of glycosaminoglycan side chains as described (2). Female BALB/c mice (National Cancer Institute, Fredrick, MD) were immunized i.p. first with cartilage PG (100 μg protein) in CFA and then with the same doses of PG on days 21 and 42 in IFA. BALB/c mice were also immunized with OVA, and lymphocytes from these OVA-immunized control mice were also used for transfer. Female SCID mice of BALB/c background (NCl/NCr.C.B-17/scid/scid), 8–12 wk of age, or young retired breeders, were purchased from National Cancer Institute and maintained under germ-free conditions. SCID mice with a “leaky” immune system were excluded from the experiments (36).

Cell isolation, depletion of T and B cells, and transfer of arthritis

Single-cell suspensions were prepared in DMEM from spleens of arthritic BALB/c mice. To maintain the uniformity and high reproducibility of transferred arthritis, donor cells were isolated from arthritic BALB/c mice within 2–3 wk after the onset of the primary arthritis, and the cumulative inflammatory score of arthritic donor mice ranged between 5.0 and 8.0 (Table I). The mononuclear cells were isolated on Lympholyte-M (Zymed Laboratories, San Francisco, CA) and used either as unseparated lymphocytes, or were depleted of specific subsets. Unseparated lymphocytes were cultured in vitro in 6-well plates with Con A (1.5 μg/ml) or LPS (25 μg/ml) for 3 days, or with cartilage PG (50 μg/ml) for 4 days in DMEM supplemented with 5% FBS (HyClone Laboratories, Logan, UT). Nonlymphoid and dead cells were removed on Lympholyte. Mononuclear cells (>99% viable after purification) were injected into recipient SCID mice i.p.

The optimum cell number, doses of cartilage PG, the route of administration (i.v. vs i.p.), and the optimum, interval between injections, including the potential effect and/or necessity of a second PG injection in T cell-depleted transfers, were determined empirically in preliminary experiments. Finally and uniformly, 2 × 10^7 unseparated spleen cells, or 1 × 10^7 unseparated, T or B cell-depleted lymphocytes or in vitro-stimulated lymphocytes were injected i.p. on days 0 and 7 in all transfer experiments presented in this paper. Cells were injected together with 100 μg PG on day 0, but in vitro-stimulated cells were transferred without PG or mitogen.

Clinical assessment of arthritis

Donor BALB/c and recipient SCID mice were examined daily for clinical symptoms of arthritis. A standard scoring system (2, 12, 37), based upon swelling and redness of each paw (ranging from 0 to 4 of each paw, thus resulting in a possible maximum severity score of 16), was used for the assessment of disease severity. Typically, in the primary form of PGIA, BALB/c mice developed swelling and redness in one or more limbs 7–14 days after the third injection with PG and adjuvant (1, 2, 37). In the transfer system, recipient SCID mice developed a more uniform disease involving nearly all peripheral joints.

Blood samples without or with 10 U of heparin (Sigma-Aldrich)/100 μl blood were collected from the retroorbital venous plexus of recipients at different time points during cell transfer experiments. Limbs were dissected on block, fixed, decalcified, sectioned, and the sections were stained with H&E for histopathological examination.

Measurements of Ag-specific Abs and T cell responses

During transfer experiments, sera were collected from SCID mice twice a week, and both sera and spleen cells were collected at the end of experiments (usually on wk 7). PG-specific Abs were measured by ELISA. MaxiSorp immunoplates (Nunc, Roskilde, Denmark) were coated with human or mouse cartilage PGs (0.1 μg protein/well), and the free binding sites were blocked with 1% fat-free milk in PBS (4, 7, 8). Sera were applied at increasing dilutions, and isotypes of PG-specific Abs were determined using peroxidase-conjugated rat anti-mouse IgG1, or IgG2a and IgG2b (Zymed Laboratories) and rat anti-mouse IgG3 (Accurate Chemical and Scientific, Westbury, NY) secondary Abs as described (8, 11). Serum Ab levels were calculated relative to the corresponding mouse IgG isotype standards (all

Donor Cells From BALB/c Mice

<table>
<thead>
<tr>
<th>Donor Cells From BALB/c Mice</th>
<th>Donor Cells with</th>
<th>Conjunction of</th>
<th>Incidence of Arthritis</th>
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<td>0/10</td>
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<td>16/16</td>
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<td>T cells</td>
<td>hPG (twice)</td>
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*The cumulative arthritis score of donor BALB/c mice was 7.2 ± 2.1 (n = 32), and the Ag (PG)-specific T cell proliferation (SI = mean ± SD) of arthritic donor BALB/c mice was 2.2 ± 0.3 to hPG and 2.0 ± 0.7 to mPG (Fig. 3, first paired columns).

N/A, none applicable. Animals used in preliminary experiments are not incorporated in Table I.

1 OVA-immunized mice exhibited an SI of 6.8 ± 2.1, but no OVA-specific T cells were recovered from SCID mice on wk 7.
from Zymed Laboratories). Ag-specific T cell responses were measured in quadruplicate samples of spleen cells (3 × 10^7 cells/well) cultured in the presence of 25 μg PG protein/ml. T cell proliferation was assessed on day 5 by incorporation of [3H]thymidine (5, 38). The Ag-specific T cell response was expressed as stimulation index (SI), a ratio of incorporated [3H]thymidine (cpm) in Ag-stimulated cultures relative to cpm measured in nonstimulated cultures (2, 5). Ag-specific IFN-γ, IL-4, and IL-10 productions were measured in cell culture supernatants (3 × 10^7 cells/ml) on day 4 using capture ELISA methods (BD PharMingen) as described (8).

Flow cytometry

Cell surface markers and intracellular cytokines were analyzed by flow cytometry. Biotinylated, FITC-, PE- or CyChrome-labeled mAbs to cell surface markers (CD3, CD4, CD8, CD45R/B220, and CD19) were purchased from BD PharMingen, and cells were stained according to standard protocols (9, 38, 39). Cytokine-producing cells were determined using intracellular cytokine staining kit (BD PharMingen) with minor modifications (40). Splenocytes or freshly isolated lymphocytes from spleens or joint-draining lymph nodes were stimulated with PMA (50 ng/ml) and ionomycin (10 μM) in DMEM supplemented with 10% FBS for 8 h. To block the subsequent cytokine release, monensin was added to the cells at 3 μM final concentration 1 h after the addition of PMA and ionomycin (41). Cells were washed in 10% FBS-containing DMEM, and after initial surface Ag staining with biotinylated-anti-CD4 Ab and streptavidin-Cy-Chrome, cells were fixed and permeabilized with saponin according to the manufacturer’s instruction. Intracellular cytokines were stained with FITC-anti-IFN-γ and PE-anti-IL-4 mAbs for 30 min at 4°C. Cells were washed and fixed in 2% formalin. Intracellular cytokine levels were measured using a FACScan instrument (BD Biosciences, Mountain View, CA), and analyzed using CellQuest software (BD Biosciences).

Statistical analysis

Statistical analysis was performed using SPSS v7.5 (SPSS, Chicago, IL). The Mann-Whitney and Wilcoxon tests were used for intergroup comparisons. Significance was set at p < 0.05.

Results

Adoptive transfer of PGIA to SCID mice using spleen cells

To test whether spleen cells from arthritic animals could transfer arthritis into SCID mice, unseparated spleen cells from arthritic donor BALB/c mice were injected into SCID mice. The number of animals, groups, and types of transfer experiments are summarized in Table I. When SCID mice were injected simultaneously with spleen cells and PG, or with in vitro PG-stimulated spleen cells, all recipient animals developed arthritis by day 41 (Fig. 1A). The onset of arthritis was delayed and the severity was slightly lower in SCID mice that received in vitro PG-stimulated splenocytes than in those injected with cells and PG simultaneously (Fig. 1). However, the clinical appearance of the disease (Fig. 2A) and the histopathology of acutely inflamed joints were similar (Fig. 2C), and indistinguishable from those described in primary PGIA (1, 2).

In the primary form of PGIA, BALB/c mice developed arthritis 10–15 days after the third PG injection (approximately on wk 9–10 after the first injection), and full-blown arthritis was reached 4–6 wk later with a maximum cumulative score of 7.2 ± 2.1. In comparison with other arthritis models, the disease (primary PGIA) was progressive, and once a joint or paw became inflamed, it progressed with remissions and flare-ups until the cartilage was completely destroyed in the affected joint (1, 2). The clinical picture in BALB/c mice was heterogeneous, as different stages of the disease, from acute inflammation to severe ankylosis and joint deformities, were seen in the same animal. In contrast, SCID mice with transferred arthritis developed acute inflammatory arthritis, which reached the maximum cumulative score of 12.3 ± 3.1 within a couple of weeks (Fig. 1B), and more importantly, essentially all peripheral joints became inflamed almost synchronously (data not shown). The earliest onset of arthritis (inflammation) was first seen as massive redness and swelling of the interphalangeal, metacarpo-, and metatarsophalangeal joints (Fig. 2, A and C).

In contrast to SCID mice injected with either in vivo or in vitro PG-stimulated spleen cells from arthritic donors, mice that received spleen cells alone (without PG), PG Ag alone (without cells), or spleen cells stimulated in vitro with LPS and/or Con A (Fig. 1) did not show any symptoms of inflammation. SCID mice injected with PG and spleen cells from BALB/c mice that had been immunized with irrelevant Ag (i.e., OVA) did not develop arthritis either (Table I). These results suggest that Ag-specific lymphocyte stimulation is required for adoptive transfer of PGIA to SCID mice.

Autoantigen (mPG) promotes adoptive transfer of PGIA

It is strongly believed that cross-reactive immune responses between the immunizing human and mouse (self) cartilage PGs are involved in the induction of PGIA (3, 5). Thus, mPG present in the joint is thought to be an ultimate target of autoimmune attacks in PGIA. Although lymphocytes from inflamed joint-draining lymph nodes exhibit a stronger T cell response to mPG than those from spleen (5, 38), no direct evidence supports the role of mPG as a

FIGURE 1. Incidence (A) and severity (B) of adoptively transferred PGIA in SCID mice. Freshly isolated spleen cells from arthritic BALB/c mice along with 100 μg of PG protein or in vitro PG-stimulated spleen cells (1 × 10^7 cells/recipient) were injected i.p. into SCID mice on days 1 and 7 ( ¡ ). A, Arthritis developed in all SCID mice (100% incidence) that received cells stimulated with PG either in vivo (△) or in vitro (©). B, The maximum severity scores of arthritis in SCID recipients (12.3 ± 3.1 and 9.7 ± 2.7) were comparable with those in donor BALB/c mice (7.2 ± 2.1). Cells from the same donor mice, injected alone (without PG) or after culture in vitro with Con A or LPS, or when these in vitro-stimulated cells were mixed, did not provoke joint inflammation in recipient SCID mice. Data from these negative groups are combined and indicated by a shared symbol (†). Figure summarizes results of three independent transfer experiments performed on 12 SCID mice in each positive (arthritic), and 8–10 animals in each negative control group. Arrowhead indicates the coinjection of PG in the standard (△) transfer group.
target molecule. To investigate the role of mPG in PGIA, we performed transfer experiments using mPG for challenge. When SCID mice received spleen cells from arthritic BALB/c donors together with mPG, arthritis developed in all recipients with an onset time and severity (cumulative score 11.3) significantly higher than that measured in PG-stimulated spleen cell cultures of arthritic donor BALB/c mice. Arthritic groups are indicated with “A” inside the column. Levels of significances (*, p < 0.05; **, p < 0.01) are shown comparing hPG- or mPG-induced in vitro T cell proliferation in arthritic donor BALB/c mice with the response (mean SI ± SD) measured in spleen cell cultures of recipient SCID mice (n = 10 per group).

**FIGURE 2.** Joint inflammation and paw swelling in SCID mice with adoptively transferred PGIA. All mice that received PG-stimulated spleen cells developed arthritis between days 10 and 40 after the first cell transfer (Fig. 1A). A, An acutely inflamed hind paw (<2 days after onset) is shown with the contralateral (left) paw with initial swelling on one digit only. Inflammation (swelling and redness) was usually first localized in small (interphalangeal and metatarso-phalangeal) joints of paws. Inflammation occasionally caused severe blood congestion and cyanosis (seen as dark phalanges of the digits on the right hind paw). In nonarthritic groups, all SCID mice exhibited normal joint structures (B), whereas arthritic mice showed robust inflammatory cell infiltration accompanied by cartilage erosion (C). Histologically, joint inflammation was similar to that described in BALB/c mice with primary PGIA (1, 2, 13). For comparison, histology sections from corresponding areas of metatarso-phalangeal joints of control (negative) and arthritic SCID mice were selected for B and C.

Ag-specific T cell response, Ab production, and lymphocyte subsets in recipient SCID mice

We have shown that Ag (PG)-specific T cell response was detected only in arthritic animals (Fig. 3). Interestingly, spleen cells from arthritic SCID mice consistently exhibited higher responses to PG than those isolated from arthritic donor BALB/c mice (Fig. 3, first paired column vs third to fifth paired columns). Based on the results of in vitro stimulation with mPGs and hPGs (Fig. 3), and the results shown on the side diagram of Fig. 4, we estimated that the frequency of PG-specific T cells were approximately four times higher in arthritic SCID mice than in arthritic BALB/c donor mice, suggesting an Ag-specific expansion of these cells in the recipient SCID mice. Remarkably, SCID mice that received spleen cells alone, or spleen cells stimulated in vitro with either Con A or LPS, completely lost their Ag (PG)-specificity by the end of the 7-wk experimental period.

Serum levels of anti-PG Abs (against both mPGs and hPGs) peaked on days 8–16, usually a few days before the onset of arthritis (Fig. 4, B and C). IgG1 levels remained at the plateau, whereas IgG2a and IgG2b concentrations dramatically declined after the onset of arthritis, and this was consistent in all arthritic animals. Similar to arthritic donor BALB/c mice (3, 5, 11), IgG1 Abs to either mPG or hPG were higher than IgG2a Abs in recipient SCID mice (Fig. 4). The IgG2b anti-PG Ab levels were lower than the IgG2a, and the kinetics of the anti-PG IgG2b were the same as shown for IgG2a (Fig. 4). IgG3 isotype anti-PG Abs were technically under the detection level in arthritic SCID mice. PG-specific Abs were detected at very low levels, or not at all, in sera of nonarthritic SCID mice (Fig. 4, A, D, and E). In conclusion, these results (Figs. 3 and 4) demonstrated that PG-specific T cell responses and serum Abs together appeared to correlate best with the onset and severity of arthritis, as neither T cell responses (Fig. 3) nor Abs (Fig. 4) were detected in the negative (nonarthritic) control groups. Lymphocyte subsets within SCID mice did not discriminate between arthritic and nonarthritic status (see marginal bar graphs in Fig. 4, A–C), except for those mice that received Con A- or LPS-stimulated cells (Fig. 4, D and E). The ratios of CD4+ to CD8+ T cells, or CD3+ T cells to B cells, measured before the injection (day 0) were similar to the ratios determined at the end of experiments (day 49; Fig. 4).

**FIGURE 3.** Ag (PG)-specific T cell proliferation in SCID mice with adoptively transferred PGIA. Ag-specific T cell stimulation in the presence of hPG (■) and mPG (▲) was determined by using spleen cells from arthritic donor BALB/c (before the transfer) or recipient SCID mice (at the end of experiment on day 49). The PG-specific T cell proliferation in arthritic SCID recipients (except those cojected with mPG) was significantly higher than that measured in PG-stimulated spleen cell cultures of arthritic donor BALB/c mice. Arthritic groups are indicated with “A” inside the column. Levels of significances (*, p < 0.05; **, p < 0.01) are shown comparing hPG- or mPG-induced in vitro T cell proliferation in arthritic donor BALB/c mice with the response (mean SI ± SD) measured in spleen cell cultures of recipient SCID mice (n = 10 per group).

The effects of T and B cell depletion upon arthritis transfer

We have shown that while the transfer of arthritis into lethally irradiated and bone marrow-transplanted syngeneic mice required both T and B cells, Ab alone was insufficient to transfer the disease (13). To elucidate the roles of different immune cells and Abs in disease development, T or B cell-depleted donor lymphocyte populations were injected into SCID recipients.

SCID mice injected with B cell-depleted donor T lymphocytes developed arthritis without delay (Fig. 5A) or reduction in severity (Fig. 5B). Although the B cell-depleted donor T lymphocytes used for transfer contained only 0.5–1% CD45R/B220+ cells (Fig. 6A, panel 1; also confirmed by anti-CD19-Ab staining), the percentage of B cells reached 12–15% in arthritic SCID mice by the end (day...
FIGURE 4. Serum anti-PG Ab levels in SCID mice throughout the transfer experiments, and the percentage and ratios of lymphocyte subsets before (day 0) and at the end (day 49) of the transfer experiments. Abs to hPGs and mPGs (both IgG1 and IgG2a) are shown in SCID mice injected with: A, Spleen cells without PG Ag; B, Spleen cells coinjected with hPG; or C, mPG; D, Spleen cells stimulated in vitro with Con A; or E, LPS. In vitro-stimulated cells were separated on Lympholyte before the i.p. injection. Ab levels against the immunizing hPG or mPG were measured by ELISA. Serum levels of IgG1 (●) and IgG2a (○) isotypes (in milligrams per milliliter) are indicated on the left and right y-axes of each panel, respectively. The percentage of transferred CD3⁺ T and CD45R/B220⁺ B lymphocytes are shown as bar graphs at both sides of each panel. The left-side bars show the percentage of cells before transfer, whereas columns at the right show cell percentage measured at the end of the transfer experiments. Within the T cell columns, the open area represents the percentage of CD4⁺ T cells, and the closed bars indicate the percentage of CD8⁺ T cells. The proportions of lymphoid subsets after transfer were determined for each animal, and the results represent the means ± SD (n = 10–12 animals). B and C. Horizontal broken line arrows illustrate the time frame from the earliest onset to the time point when all mice developed arthritis. Arrows at bottom of the panel show the time of cell transfer (for all groups), whereas arrowheads on B and C indicate PG coadministration.

49) of the transfer experiment (Fig. 6A, panel 2). In contrast, when T cell-depleted donor lymphocytes were transferred with PG (Fig. 6A, panel 3), no arthritis developed in any SCID mice (n = 18) after an extended (10 wk) observation period (Fig. 5A, □). The spleens of these nonarthritic recipients contained <2% (original ratio) CD3⁺ T cells. To explore an arthritogenic potential of this small population of T cells, in the next sets of transfer experiments, an extra 100 μg PG Ag (without cells) was injected i.p. into these nonarthritic SCID mice on day 21 (Fig. 5). All SCID mice (n = 18) that received T cell-depleted lymphocytes developed arthritis within 5–12 days after the second Ag challenge (Fig. 5A), although the disease was less severe (Fig. 5B) than in animals receiving unseparated (Fig. 1B) or B cell-depleted lymphocytes (Fig. 5B). The ratio of CD3⁺ T cells reached 7–10% by wk 7 (Fig. 6A, panel 4) in SCID mice that were challenged with an additional PG injection without cells (Fig. 5B, □). Remarkably, 92–96% of the recovered CD3⁺ cells showed CD4⁺ phenotype (Fig. 6B, panel 4), and T cells harvested from this group of mice exhibited an unusually high PG-specific response (SI: 6.9 ± 1.2).

Intracellular cytokine staining revealed that the Th1/Th2 ratio (calculated as ratios of IFN-γ/IL-4) in CD4⁺ T cells) among these recovered T cells was high (Fig. 7), and frequently reached >8/1 in the arthritic SCID mice. Notably, the Th1 cell proportion within the CD4⁺ population was higher in all arthritic SCIDs than in arthritic donor BALB/c mice, underscoring the possibility of a selective proliferation of PG-specific Th1 cells in the SCID host. In support of this statement (Fig. 7), the CD4⁺ IFN-γ/IL-4 ratio was 8.2 ± 1.0 in arthritic SCID mice vs 4.3 ± 3.1 in BALB/c donors with primary arthritis, and the CD4⁺ IFN-γ/IL-10 ratio was 6.0 ± 2.2 in SCID and 2.1 ± 1.9 in arthritic BALB/c mice.
PG-specific Ab production is T cell-dependent and correlates with arthritis

The most unexpected observation was that anti-PG Abs were almost undetectable before a second PG Ag injection (Fig. 8A) in SCID mice that received T cell-depleted (i.e., B cell-enriched) lymphocytes. The second PG injection (without cells) strongly promoted the survival and expansion of CD4+ T cells (Fig. 9A) without promoting CD8+ cell recovery (Fig. 9B). This was followed by a rise in anti-PG Ab production (either to mPG or hPG, both IgG1 and IgG2a isotypes; Fig. 8A). Soon after the appearance of anti-PG Abs, mice developed arthritis (Fig. 5). These results imply that B cells require CD4+ (most likely Th1) cells for cooperation in arthritis induction. This finding seems to be supported by an even more intriguing observation: Ab production, although restricted to IgG1 isotype, showed a complete "recovery" by days 8–12 in SCID mice injected with B cell-depleted cells (Fig. 8B). As described above (Fig. 6A, panel 1), the contaminating CD45R/B220+ cells were consistently <1% in B cell-depleted cell suspensions (i.e., the number of CD3+ T cells was at least 100–150 times higher than the number of B lymphocytes). Based on the kinetics of serum anti-PG Ab production in SCID mice injected with B cell-depleted cells (Fig. 8B), one would expect a rapid B cell recovery in arthritic SCID mice. However, flow cytometric analysis showed no significant increase in B cell numbers (Fig. 9C).

Discussion

PGIA is a genetically restricted autoimmune disease in which Th1 cells play a dominant role (3, 10, 11, 16), and disease susceptibility
is linked to both MHC (H-2d or H-2K) and non-MHC genetic components (7, 8). The disease can be transferred by coinjection of B and T cells into irradiated syngeneic mice (13, 17). However, the incidence and severity of the disease are lower than in the donor BALB/c mice and the individual differences are relatively high among the recipients. To simplify the transfer system for studying the functions of T and B cells and/or Abs, we used BALB/c scid/ scid mice as recipients, taking advantage of both the immune deficiency and the matching genetic background (42). To date, all arthritis transfer studies using either animal (31–33) or human donor cells from patients with rheumatoid arthritis (43–45) to SCID mice, the donors and recipients differed in both MHC- and non-MHC-related genetic backgrounds. Under these conditions, the transferred autoimmune disease could be complicated by alloantigen recognition. Therefore, not surprisingly, when lymphoid cells from arthritic DBA/1 × B10.Q F1 mice were transferred into BALB/c-SCID mice, relapsing arthritis was observed over a 250-day period (46), which might be the results of graft vs host disease superimposed on arthritis.

To address the role of T and B cell cooperation and the functions of T cell subsets in arthritis induction, we injected genetically matched SCID mice with different subsets of arthritic BALB/c-derived lymphocytes. In agreement with our previous observations (13), the PG-specificity of transferred T or B cells, which was initially present, disappeared after a few weeks without Ag stimulation (Fig. 3), and nonspecific stimulation (with Con A and/or LPS) of either T or B cells derived from arthritic BALB/c donors failed to transfer the disease (Fig. 1). Therefore, Ag-specific T cell activation seems to be required for successful transfer of arthritis. However, this was expected, as all autoimmune disease transfer experiments required an Ag-specific stimulation of donor cells applied either before (in vitro stimulation) or at the time (coinjection) of cell transfer (13, 31, 47). A single dose (100 μg) of PG coinjected with spleen cells into SCID mice (a dose of PG without adjuvant, which otherwise did not induce detectable immune responses in BALB/c mice; Refs. 1, 3, and 6), or in vitro stimulation of spleen cells with PG, was absolutely crucial for activation of primed donor lymphocytes and the transfer of a clinically severe disease (Fig. 1). In terms of the source of PG, there were no or little differences in Ag-induced T cell stimulation in the donor arthritic BALB/c mice, and T cell recovery and arthritis induction was successful when either hPG or mPG was used. We can postulate that the amount of PG or PG fragments released during the normal turnover of cartilage in SCID mice at the time of transfer might be insufficient to support an Ag-specific T cell proliferation or lymphocyte recruitment to the synovial joints. As the mPG was as effective as the human in arthritis induction, this was a strong evidence that mPG was a target immunogen in vivo, and was critically involved in the progression of autoimmunity and local inflammatory reactions in PGIA.

Restoration of lymphocyte balance can be accomplished using either normal (naive) or Ag-specific T cell populations in severe T cell deficiency syndromes which develop following whole-body
irradiation or chemotherapy, or in a severe combined immune-deficiency disorder (called “bare lymphocyte syndrome”; Refs. 48 and 49). In normal T cell development, as a result of positive and negative selection, the postthymic repertoire of mature T cells consists of lymphocytes reactive with MHC-associated foreign peptides, but tolerant of self-peptides (21, 24). The long-term survival of mature CD4+ T cells requires continuous, albeit covert, signals through the TCR. These covert (weak) signals that are transmitted by self-MHC/self-peptides (25) acquire a critically important function when the size of the T cell pool is dramatically reduced (e.g., after chemotherapy). Similar conditions occur when relatively low numbers of T cells (1 × 10^3) are transferred into syngeneic nude or SCID rodents leading to a massive expansion of donor cells and restoration of T cell homeostasis (18, 19).

A number of studies investigated the function of T and B cells in the development of various autoimmune diseases using transfer of lymphocyte subsets (32, 50, 51). We did not expect that the very low proportion of contaminating T or B lymphocytes, within this population even much fewer Ag-specific cells, could modify the outcome of disease transfer. Toward the end of these experiments, it appeared that either T or B cell-depleted lymphocytes from arthritic donors could transfer arthritis into SCID mice, albeit B cells seemed to be less critical, as arthritis was transferred without delay in their absence (Fig. 5).

We found remarkable changes in the recovery of “contaminating” cells during the course of arthritis adoptively transferred to syngeneic SCID mice. It became evident that the very small number of the Ag-specific T or B cells in the 0.5–2.0% transferred T cell population even much fewer Ag-specific cells, could modify the outcome of disease transfer. Toward the end of these experiments, it appeared that either T or B cell-depleted lymphocytes from arthritic donors could transfer arthritis into SCID mice, albeit B cells seemed to be less critical, as arthritis was transferred without delay in their absence (Fig. 5).

We found remarkable changes in the recovery of “contaminating” cells during the course of arthritis adoptively transferred to syngeneic SCID mice. It became evident that the very small number of the Ag-specific T or B cells in the 0.5–2.0% transferred contaminating cell population served as precursors of an expanding immune system in recipient SCID mice, but only in the presence of relevant autoantigen (mPG). PG-specific B cell recovery was faster than T cell recovery, and the T cell recovery was limited to the CD4+ lymphocytes of primarily Th1 phenotype. Although the initial overall 1–2% T cell content in T cell-depleted populations did not change for weeks (Fig. 9), these precursor cells gave rise to increasing numbers of Ag-specific Th1 cells (Fig. 6B, panel 4), especially after the second PG challenge on day 21 (Figs. 5 and 9A). Concomitantly with the expansion (rescue) of Ag (PG)-specific Th1 cells, B cells were able to differentiate to plasma cells (52) and began to produce PG-specific Abs (Fig. 8A); this was followed by the development of arthritis in SCID mice. In this mechanism, i.e., arthritis induction in SCID mice with transferred cells, Ag-induced B cell stimulation and proliferation, the PG-specific Ab production and the Ag presentation by B cells might equally be involved. In previous experiments, we found that B cells from naive (none immune) BALB/c mice presented PG Ag several-fold more effectively than peritoneal macrophages, and B cells from PG-immunized mice proved to be at least 1000 times better APCs than macrophages or adherent mononuclear spleen cells from the same immunized animal (5). Therefore, it seems to be very likely that a small number of contaminating B cells in the B cell-depleted cell transfer system were capable of presenting PG Ag to T cells much more effectively than the host’s APCs. A second injection of PG Ag (Fig. 5) might amplify this B cell function accompanied with a concomitant phenomenon, dramatically increasing the PG-specific Ab production (Fig. 8A). Although this remains an unsolved question and requires additional studies focusing on the Ag presenting function of B cells, the major hallmark of this observation is that Ag (PG)-specific B cells were unable to produce (auto)Abs, nor induce arthritis, or be involved in arthritis induction, in the absence of Ag-specific CD4+ cells.

Expansion of mPG-specific syngeneic CD4+ Th1 cells in SCID mice is consistent with the results of other studies that placed the focus on the restoration of the T cell homeostasis (21–25). However, the recovery of pathogenic CD4+ Th1 cells from an extremely small population in an autoimmune transfer system is a novel and unique observation within this study, as restoration of Ag-specific T cells was not monitored during the development of the autoimmune disease in any of the previous transfer experiments. The most unexpected observations were that B cells, when T cells were depleted, did not produce PG-specific Abs unless SCID mice received a second PG injection (Fig. 8A) and, reciprocally, high levels of serum anti-PG Abs were found in SCID mice with transferred arthritis that received B cell-depleted T lymphocytes (Fig. 8B).

The critical role of T cells in arthritic processes is unquestionable, although the exact functions of various subsets have not been elucidated. CD8+ cells were generally defined as suppressor cells in autoimmune diseases (33, 53), and the ratio of CD8+/CD4+ cells was low at the onset of both CIA (53) and PGLA (38). However, CD8+ cells were proposed to function as effector cells in experimental allergic encephalomyelitis (51). In T cell-depleted cell transfer experiments, Ag-specific T cell proliferation resulted in CD4+ lymphocyte recovery without restoration of CD8+ lymphocytes. This might be explained by the observation that CD4+ cells could outlive CD8+ cells in long-term SCID transfer experiments (36). However, when we injected B cell-depleted (T cell-enriched) mixed (CD4+ and CD8+ ) T cell populations, or unseparated lymphocytes into SCID mice, the CD4+/CD8+ ratios did not change for up to 7 wk (Fig. 4A). As the total number of CD8+ cells did not decrease during the experimental period, (i.e., no selective CD8+ cell death was observed; Fig. 9B), the expansion of CD4+ cells must be due to a marked increase in the Ag-specific CD4+ cell population. Remarkably, these SCID mice showed high proportion of CD4+ Th1 cells, and SCID mice injected with T cell-depleted lymphocytes exhibited unusually high Th1/Th2 ratios (Fig. 7). These findings clearly indicate that a very low number of PG-responder CD4+ T cells in SCID mice, under the “pressure” of covert signals from self-MHC/self peptide supported with overt signals from coinjection with PG, underwent positive selection and subsequent expansion recovering the “arthritogenic” phenotype of the immune system in syngeneic SCID-BALB/c background.

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