Transfer of the Inflammatory Disease of HLA-B27 Transgenic Rats by Bone Marrow Engraftment

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Summary

We have previously produced lines of rats transgenic for HLA-B27 and human β2-microglobulin (hβ2m) that develop a progressive inflammatory disease sharing many clinical and histologic features with the B27-associated human spondyloarthropathies, including gut and male genital inflammation, arthritis, and psoriasiform skin lesions. Other transgenic lines that express lower levels of B27 and hβ2m remain healthy. To investigate the cellular basis for the multisystem inflammatory disease in these rats, we transferred lymphoid cell populations from disease-prone transgenic lines to irradiated disease-resistant transgenic and nontransgenic recipients. In recipients of cells from two different disease-prone lines, successful transfer required engraftment of bone marrow cells. Transfer of disease with fetal liver cells suggested that neither mature effector cells nor active disease in the donors was necessary for induction of disease in the recipients. Remission of the spontaneous disease in irradiated transgenic rats was induced by engraftment of nontransgenic bone marrow. These results suggest that the expression of HLA-B27 in bone marrow-derived cells alone is sufficient for the development of B27-associated disease, and that disease transfer requires engraftment of a bone marrow precursor cell for which mature cells in spleen or in lymph node cannot substitute.

Several human diseases show association and genetic linkage with class I or II alleles of the HLA complex (1). Among the most striking of these associations is that of the class I allele HLA-B27 with the disorders termed spondyloarthropathies, in which there is inflammatory involvement of axial and peripheral joints, gastrointestinal and genitourinary tracts, skin, eye, and heart (summarized in reference 2). For most HLA-associated disorders, it has not been determined whether the associated alleles participate directly in disease pathogenesis. However, for the HLA-B27-associated spondyloarthropathies, the development of similar disease in transgenic rats expressing HLA-B27 has provided strong evidence for direct participation by the B27 molecule. Rats of two transgenic lines, 21-4H and 33-3, each hemizygous for a transgene locus bearing genes encoding HLA-B*2705 and hβ2m,1 uniformly develop a progressive inflammatory disease (2, 3). The major features of this disease include chronic diarrhea resulting from gastrointestinal inflammation, peripheral and axial arthritis, psoriasiform skin and nail changes, and male genital inflammation. In a third line, 21-3, similar disease develops in rats that are homozygous for the transgene locus, whereas the hemizygotest remain healthy. Rats that are either hemi- or homozygous for the transgene loci of the 21-4L and 25-6 lines, which bear fewer copies of the transgenes than the 21-4H, 33-3 and 21-3 lines, have remained uniformly healthy (3). All five lines bear the same genomic HLA-B*2705 and hβ2m constructs, differing with respect to the transgenes only in the numbers of copies and levels of expression (2, 3).

Because the histologic appearance of the lesions suggested strongly that the disease in rats is immune mediated, and because an immune pathogenesis is also suspected in most of the B27-associated disease manifestations in humans (4, 5), we sought to identify cells of the immune system that could transfer the disease induced by the B27 transgene. Here we report the results of these cell transfer experiments.

Materials and Methods

Animals. The transgenic rat lines 21-4L, bearing 6 copies each of the HLA-B*2705 and hβ2m genes; 21-4H, bearing 150 copies of the B*2705 gene and 90 copies of the hβ2m gene; and 33-3, bearing 55 copies of the B*2705 gene and 66 copies of the hβ2m gene, were produced and maintained in our animal colony as previously described (2). Nontransgenic LEW and F344 rats were pur-
chased from Charles River, Inc. (Wilmington, MA). In September 1992, after many of the experiments involving the 21-4H and 21-4L lines had been carried out, we became aware that Charles River, Inc., had provided us with LEW breeding stock in 1991 that was contaminated with genes from an outbred line. Typing by hemagglutination for three RBC alloantigens systems (RT2, RT3, and RT8) (6, 7) and for MHC haplotype (RT1) indicated that some rats of the 21-4L line carried a non-LEW RT2 allele, but all were homozygous for the expected LEW alleles at RT1, RT3, and RT8. Some rats of the 21-4H line were found to carry a non-LEW RT1 haplotype (RT7), but all were homozygous for the expected LEW alleles at RT2, RT3, and RT8. Rats of the 33-3 line showed only F344 alleles at four out of 14 linked loci typed serologically, and also at 10 out of 14 unlinked loci identified by microsatellite DNA polymorphisms, as described (8). The 33-3 line was therefore likely to be authentically inbred on the F344 background.

Cell Suspensions. Single cell suspensions were prepared from bone marrow (BM), spleen, peripheral or mesenteric LN, or blood, as described (9). Fetuses from time-mated pregnant females were harvested at various gestational points (presence of vaginal sperm taken as day 0). Single cell suspensions of fetal liver (FL) cells were made by disruption of minced fetal liver with a Pasteur pipette in 0.5 ml Dulbecco's PBS and filtration through nylon mesh before counting and injection. The HLA-B27 transgene status of the fetuses was determined by dot-blot hybridization of genomic DNA, as described (2).

Cell Transfers. Recipients were either sublethally (300, 500, or 750 rad) or lethally (950, 1,050, or 1,350 rad) irradiated at 70 rad/min from a 137Cs source (Gammacell 40; Atomic Energy of Canada, Ltd., Kanata, Ont., Canada). To reconstitute recipient BM after lethal irradiation (3), nonirradiated (NI) or chilling-irradiated BM was infected intravenously with the external jugular vein under ether anesthesia. Single cell suspensions prepared from individual FL were injected into individual recipients. Lethally irradiated nontransgenic F344 recipients of 33-3 LN cells also received a rescuing inoculum of 3 × 10^7 FL cells from day 16 nontransgenic syngeneic fetuses.

Clinical Evaluation. Recipients were monitored five times per week for chronic diarrhea, arthritis, and skin changes developing after or persisting beyond the first month after irradiation. The severity of diarrhea, arthritis, and skin changes was given a daily severity score of 0-4 each. The average daily total of these scores was calculated each week, with a maximum of 12, and the average of these weekly scores for each rat for the first 3 mo after the onset of disease was used as an index of disease severity. In all passive transfer experiments, control rats were observed past the time of latest onset of disease in affected rats. Fatal cachexia beyond the first month after irradiation was considered a consequence of the inflammatory disease manifestation, since in preliminary studies, the radiation doses termed lethal (950-1,350 rad) were uniformly fatal within 3 wk in unirradiated rats.

Flow Cytometry Analysis. Single and two-color flow cytometry were carried out as described (3), with some modifications. The following mouse mAb and their specificities were used, references for which are cited in (3): B1.23.2, IgG2a, monomorphic HLA-B and -C; R73, IgGl, rat TCR-α/β C region; W3/25, IgGl, rat CD4; OX8 IgGl, rat CD8α/β chain; OX33, IgGl, B cell-specific epitope of rat CD45; and OX19, IgGl, rat CD5. The mAb 3.2.3, IgGl, recognizing NKP-P1, a rat NK cell-specific surface protein (10), and OX-42, IgGl, recognizing rat C3b receptor (11) were also used. Irrelevant IgGl and IgGl mAb served as negative controls. All procedures were carried out at 4°C in DPBS containing 4% FCS and 0.05% sodium azide (staining buffer). For single color direct fluorescence, cells were preincubated 20 min in 50 μl staining buffer containing 20% rat serum before addition of 50 μl staining buffer containing directly conjugated FITC-B1.23.2 at twice the saturating concentration for a 30-min incubation. For two-color staining, cells were incubated with mAb in the following sequence, with two washes after each incubation: relevant IgGl mAb, FITC-conjugated rat anti-mouse IgGl (Zymed Laboratories, Inc., S. San Francisco, CA), biotinylated B1.23.2, PE-conjugated streptavidin (Pierce, Rockford, IL). For single color indirect fluorescence, cells were incubated with biotinylated B1.23.2 followed by PE-conjugated streptavidin. After two washes and filtration through nylon mesh, flow cytometry was carried out as described (3).

Histopathology and Immunocytochemistry. These were carried out as described (2, 3). In addition to the mAb listed above, HC-10, mouse IgG2b, specific for HLA-B H chain (12), was also used for immunocytochemistry.

Results

Transfer of Disease to Transgenic 21-4L Rats. We initially assumed that the presence of HLA-B27 in nonhematopoietic cells within the target tissues of the disease, as well as on hematopoietic cells, might be important for disease expression. Therefore, rats of the disease-resistant B27 transgenic 21-4L line were used as recipients for cell suspensions from sick donor rats of the 21-4H disease-prone line (Table 1). Transfer of BM cells, alone or mixed with spleen cells, to sublethally irradiated (500-750 rad) recipients was associated in 11 of the 18 cases with persistent diarrhea starting 2-5 wk after transfer (group 1A, i.e., group A, Table 1). 10 recipients also later developed skin or nail changes resembling the spontaneous disease, and one recipient developed arthritis. In this first group of recipients, the number of BM cells injected ranged from 0.75 to 9 × 10^6 per recipient with no clear correlation between cell dose and the incidence or severity of disease in the recipients. In most subsequent experiments, a standard dose of 10^6 BM cells was used. After lethal irradiation (1,050 rad) and transplantation of 21-4H BM, 12 out of 12 21-4L recipients developed disease (group 1E) that was earlier in onset and more severe than that seen in the sublethally irradiated 21-4L recipients. These results indicated a significant influence of the radiation dose on the efficacy of disease transfer.

Histologic study of four of the sick recipients after 4-18 wk of disease showed mucosal inflammation diffusely distributed throughout the colon in all four rats and more focally distributed in the small intestine and stomach. Lesions were similar to those of the spontaneous disease (2), consisting mainly of mononuclear infiltration of the lamina propria, crypt epithelial hyperplasia, and loss of mucin-containing cells (Fig. 1 A). In the more severe cases, crypt abscesses were present. No vasculitic lesions were seen. Skin (Fig. 2 A) and nail changes similar to the findings in the spontaneous disease were also present, consisting of epidermal hyperplasia and hyperkeratosis with dermal inflammation and perifolliculitis. Glossitis with histologic characteristics similar to that seen in the skin was present in three of the four recipients examined. This finding is also present in the spontaneous disease (our unpublished results). No liver abnormalities were seen in any of the four rats examined.

1608 Bone Marrow Transfer of HLA-B27-associated Disease in Rats
Table 1. Passive Transfer of B27-associated Disease to Irradiated 21-4L Rats

| Group | Donor cells | Cells injected × 10^6 (range) | With disease | Total | Diarrhea | Arthritis lesions | Skin lesions (1–4 mo) | Death | Wk of onset | Severity index |
|-------|-------------|------------------------------|--------------|-------|----------|------------------|----------------------|-------|-------------|----------------|
|       |             |                              |              |       |          |                  |                      |       | Mean ± SEM  | Mean ± SEM     |
| Sublethal irradiation (500–750 rad) |             |                              |              |       |          |                  |                      |       |             |                |
| A     | 21-4H BM    | 0.75–9                       | 18           | 12    | 11       | 0                | 10                   | 0     | 7 ± 3       | 1.7 ± 0.3      |
|       | ( + SP)*    | (0.6–3)†                     | (4)          | (3)   | (3)      | (1)              | (2)                  | (0)   | (3 ± 1)     | (3.2 ± 0.5)    |
| B     | 21-4H SP    | 2–4                          | 8            | 1     | 0        | 0                | 1                    | 0     | 22 1       |                |
| C     | 21-4H LN    | 3–5                          | 7            | 1     | 0        | 0                | 0                    | 0     | 31 1.1     |                |
| D     | 21-4L BM    | 5–9                          | 7            | 0     | 0        | 0                | 0                    | 0     | – –        |                |
|       | ( + SP)     | (2)                          | (2)          | (0)   | (0)      | (0)              | (0)                  | (0)   | (0)        |                |
| Lethal irradiation (950–1,050 rads) |             |                              |              |       |          |                  |                      |       |             |                |
| E     | 21-4H BM    | 1                            | 12           | 12    | 12       | 0                | 12                   | 0     | 9 4 ± 0     | 2.2 ± 0.2      |
| F     | 21-4H SP    | 1                            | 6            | 6     | 6        | 1                | 5                    | 0     | 7 ± 2       | 0.7 ± 0.1      |
| G     | 21-4L BM    | 1                            | 0            | 0     | 0        | 0                | 0                    | 0     | – –        |                |
| H     | Ntg LEW BM  | 1                            | 10           | 0     | 0        | 0                | 0                    | 0     | – –        |                |
|       | + SP + LN   | (1.5)                        |              |       |          |                  |                      |       |             |                |

5–23-wk-old irradiated B27/hb2m transgenic rats of the disease-resistant 21-4L line received i.v. BM, spleen (SP), or LN cells from sick donor rats of the B27/hb2m transgenic disease-prone 21-4H line, from 21-4L donor rats or from nontransgenic (Ntg) donor rats. Recipients were monitored for clinical manifestations developing after or persisting beyond the first month after irradiation. Severity index was calculated from rats showing disease.

* Notations in parentheses refer to recipients of BM cells that received SP cells along with BM cells.
† Number of SP and/or LN cells injected along with BM cells in recipients of mixed cells.

In contrast with these results observed with BM, and particularly in contrast with the severe disease seen in recipients of BM plus spleen cells (group 1A), transfer of spleen or LN cells alone to sublethally irradiated recipients had an almost negligible clinical effect. Only one of eight spleen cell recipients and one of seven LN cell recipients developed any sign of disease, and this was confirmed to alopecia beginning 22 and 31 wk, respectively, after irradiation and cell transfer (groups 1B and C). One of these two recipients was examined histologically and showed dermal perifolliculitis but no epidermal hyperplasia or inflammation. The colonic mucosa was normal. This result suggested that engraftment by BM stem cells may be required for transfer of disease. Since spleen contains a small population of hematopoietic stem cells (13), if these could be successfully engrafted, it might be expected that disease would ensue. This prediction was borne out by the finding

Figure 1. Histopathology of chronic colitis induced in disease-resistant rats after transfer of BM cells from disease-prone rats. (A) Colon of a 21-4L male recipient of 21-4H BM cells 18 wk after irradiation (750 rad) and cell transfer (group 1A). The section shows chronic colitis with hyperplasia of epithelial cells, loss of mucin-containing cells, and increased depth of crypts. Marked infiltration by mononuclear cells is present throughout the lamina propria. (B) Colon of a nontransgenic LEW male recipient of 21-4L BM cells 10 wk after irradiation (1,050 rad) and cell transfer (group 2G). The histologic appearance is normal. (C) Colon of a nontransgenic female LEW recipient of 21-4H FL cells 5 wk after irradiation (1,050 rad) and cell transfer (group 2E). Lesions are present similar to those in A. (D) Colon of a 43-wk-old 33-3 female recipient of nontransgenic F344 BM cells 18 wk after irradiation (950 rad) and cell transfer showing normal crypt size and normal cellularity in the lamina propria. The presence of submucosal fibrosis appears to be a sequela of the healing process (4–D, ×130).
that six of six lethally irradiated 21-4L recipients of 21-4H spleen cells developed overt disease (group 1F). Disease in this group was milder and later in onset than that of comparable recipients of BM, as might be expected if disease was dependent upon stem cell engraftment and if the number of transferred stem cells was lower. Sublethally and lethally irradiated 21-4L recipients of control 21-4L or nontransgenic LEW cells remained healthy throughout the 13–60-wk period of observation (groups 1D, G, and H). Flow cytometry of spleen and PBM cells at 7–35 wk after transfer showed a population with high B27 surface expression in recipients that developed disease (Fig. 3 C, fluorescence intensity >10^3), similar to a population present in the 21-4H donors (Fig. 3 A), whereas both the 21-4L recipients of 21-4L BM cells (group 1D, data not shown) and the 21-4L recipients that remained healthy after receiving 21-4H BM (group 1A, Fig. 3 D) showed a typical 21-4L pattern of B27 surface expression (Fig. 3 B).

Transfer of Disease to Nontransgenic Recipients. The results obtained with the 21-4L recipients suggested that the recipients developing disease were largely repopulated by donor-derived hematopoietic cells after irradiation. However, the role of B27 expression in recipient cells not derived from BM remained undefined. Immunohistochemistry of the lesions in animals exhibiting the spontaneous disease had consistently shown B27 expression predominantly restricted to lymphoid cells, with negligible staining for B27 on gut epithelial cells (3). This suggested that disease induction might depend on B27 expression in lymphoid cells alone. Experiments were therefore performed in which 21-4H, 21-4L, 33-3, or nontransgenic F344 BM cells were transferred to lethally irradiated, sex- and strain-matched, nontransgenic LEW or F344 rats. Recipients of 21-4L or nontransgenic F344 BM cells recovered completely from the effects of irradiation and remained healthy during an observation period of 6–77 wk after transfer (Tables 2 and 3, groups 2G and 3G, Figs. 1 B and 2 C). In contrast, all 21 LEW recipients of 21-4H BM cells and all 42 F344 recipients of >0.5 × 10^8 33-3 BM cells developed persistent diarrhea and/or skin changes within 2–29 wk (groups 2D and 3C, median of 4 and 6 wk, respectively). Arthritis occurred in a total of seven animals, six of them LEW. Fatal cachexia was common in the LEW recipients (8 of 17 within 4 mo), but occurred in only one of the F344 recipients. Histologic lesions similar to those of the spontaneous disease and of the 21-4L recipients of 21-4H BM were present in the gut (data not shown), skin (Fig. 2 B), and...
Table 2. Passive Transfer of B27-associated Disease to Irradiated Nontransgenic (Ntg) LEW Rats

| Group | Donor cells | Cells injected × 10⁶ (range) | With disease | Total disease | Diarrhea | Arthritis | Skin lesions | Death (1-4 mo) | Wk of onset Mean ± SEM | Severity index Mean ± SEM |
|-------|-------------|-----------------------------|--------------|---------------|----------|-----------|-------------|---------------|--------------------------|--------------------------|
|       |             |                             |              |               |          |           |             |               |                           |                           |
| Sublethal irradiation (750 rad) |             |                             |              |               |          |           |             |               |                           |                           |
| A     | 21-4H BM    | 1                          | 10           | 9             | 6        | 4         | 5           | 0             | 10 ± 3                   | 1.4 ± 0.2                |
| B     | 21-4H SP    | 1.5-3                      | 7            | 3             | 1        | 1         | 1           | 0             | 13 ± 5                   | 0.7 ± 0.2                |
| C     | 21-4H LN    | 2.5-4                      | 7            | 4             | 0        | 0         | 4           | 0             | 21 ± 1                   | 1.1 ± 0.2                |
|       |             |                             |              |               |          |           |             |               |                           |                           |
| Lethal irradiation (950-1,050 rad) |             |                             |              |               |          |           |             |               |                           |                           |
| D     | 21-4H BM    | 1-3                        | 21           | 21            | 20       | 6        | 20          | 8             | 4 ± 1                    | 2.9 ± 0.2                |
| E     | 21-4H FL    | 0.08 ± 0.3                 | 14           | 14            | 13       | 3        | 7           | 1             | 10 ± 1                   | 1 ± 0.1                  |
| F     | 21-4H SP    | 1                          | 4            | 3             | 3        | 0        | 3           | 0             | 14 ± 1                   | 1 ± 0.3                  |
| G     | 21-4L BM    | 1.5-2                      | 18           | 0             | 0        | 0        | 0           | 0             | -                        | -                        |
| H     | Ntg LEW FL  | 0.03-0.3                   | 18           | 0             | 0        | 0        | 0           | 0             | -                        | -                        |

7-11-wk-old Ntg LEW irradiated recipients received i.v. BM, SP, LN, or FL cells from transgenic donor rats of the disease-prone 21-4H line, from transgenic donor rats of the disease-resistant 21-4L line, or from Ntg LEW donor rats, and were monitored as described in Table 1.

arthritic joints (Fig. 2 D). On flow cytometry, BM cells from recipients of transgenic BM were predominantly B27⁺ (Fig. 4, A-C). Spleen, LN, and PBMC showed surface expression of B27 in all subpopulations of lymphoid cells examined, including cells displaying TCR-α/β, CD4, CD8, CD5, NKp-P1, and the B cell–specific epitope of CD45 (Fig. 5 and data not shown). A consistent finding was the presence of a population of donor-derived non-T cells that were B27ᵇⁿˢ h', CD4 dim in the recipients that developed disease (Fig. 5, A and B). The proportion of these cells, which display monocyte-macrophage morphology and stain with the mAb OX-42, increased with disease progression (data not shown). This was correlated with absolute monocytosis evident on blood smears of sick recipients, and also present in spontaneous disease (our unpublished observations). Immunohistochemistry of colonic lesions from nontransgenic recipients of BM cells from either 33-3 or 21-4H donors showed that the cells infiltrating the lamina propria were predominantly B27⁺ (data not shown), suggesting a direct role for donor-derived cells in the inflammatory lesions.

In comparison with the lethally irradiated LEW recipients of 21-4H BM cells, sublethally irradiated recipients showed disease of later onset and lesser severity (group 2A), similar to the pattern seen with 21-4L recipients (group 1A). In addi-

Table 3. Passive Transfer of B27-associated Disease to Nontransgenic (Ntg) F344 Irradiated Rats

| Group | Donor cells | Cells injected × 10⁶ (range) | With disease | Total disease | Diarrhea | Arthritis | Skin lesions | Death (1-4 mo) | Wk of onset Mean ± SEM | Severity index Mean ± SEM |
|-------|-------------|-----------------------------|--------------|---------------|----------|-----------|-------------|---------------|--------------------------|--------------------------|
|       |             |                             |              |               |          |           |             |               |                           |                           |
| Sublethal irradiation (750 rad) |             |                             |              |               |          |           |             |               |                           |                           |
| A     | 33-3 BM     | 1                          | 12           | 3             | 3        | 0         | 0           | 0             | 35 ± 1.6                  | ND                       |
| B     | 33-3 SP or LN | 1-1.5                      | 12           | 0             | 0        | 0         | 0           | 0             | -                        | -                        |
| Lethal irradiation (1,050-1,350 rads) |             |                             |              |               |          |           |             |               |                           |                           |
| C     | 33-3 BM     | 0.5-3                      | 42           | 42            | 42       | 1        | 18          | 1             | 6 ± 1                    | 1.5 ± 0.1*               |
| D     | 33-3 BM     | 0.12-0.25                  | 6            | 4             | 4        | 0        | 3           | 0             | 13 ± 5                   | 1 ± 0.1†               |
| E     | 33-3 SP     | 1                          | 4            | 2             | 2        | 0        | 2           | 0             | 8, 13                    | ND                       |
| F     | 33-3 LN³    | 1                          | 5            | 0             | 0        | 0        | 0           | 0             | -                        | -                        |
| G     | Ntg F344 BM | 0.85 ± 1.5                 | 20           | 0             | 0        | 0        | 0           | 0             | -                        | -                        |

7-12-wk-old Ntg F344 irradiated recipients received i.v. BM, SP, or LN cells from HLA-B27 transgenic donor rats of the disease-prone 33-3 line or from Ntg F344 donor rats and were monitored as described in Table 1.

* Mean ± SEM of 39 of 42 disease-affected recipients.
† Mean ± SEM of three of four disease-affected recipients.
³ Recipients in Group F were rescued with 3 × 10⁷ d 16 Ntg F344 FL cells injected along with 33-3 LN cells.
Figure 4. Cell surface expression of HLA-B27 on BM cells in recipients of BM or FL cells. Single color histograms are shown after gating to exclude erythrocytes. The majority of BM cells express HLA-B27 on the surface in the following disease-affected recipients: (A) lethally irradiated (1,050 rad) nontransgenic LEW female recipient of 21-4H BM cells (group 2D), 6 wk after transfer; (B) lethally irradiated (1,050 rad) nontransgenic F344 female recipient of 33-3 BM cells (group 3C), 31 wk after transfer; (D, solid trace) sublethally irradiated (750 rad) nontransgenic LEW male recipient of 21-4H BM cells (group 1A), 56 wk after transfer; (E, solid trace) sublethally irradiated (750 rad) nontransgenic LEW female recipient (group 2B), and (F, solid trace) lethally irradiated (1,050 rad) nontransgenic LEW male recipient (group 2F) of 21-4H cell populations, 18 and 34 wk after transfer, respectively; and (G) lethally irradiated (1,050 rad) nontransgenic LEW male recipient of 21-4L BM cells (group 2G), 59 wk after transfer. In contrast, the proportion of BM cells expressing B27 is much lower or negligible in the following healthy recipients; (D, dashed trace) sublethally irradiated (750 rad) nontransgenic LEW female recipient of 21-4H BM cells (group 2A), 24 wk after transfer; (E, dashed trace) sublethally irradiated (750 rad) nontransgenic LEW male recipient of 21-4H BM cells (group 2B), and (F, dashed trace) lethally irradiated (1,050 rad) nontransgenic LEW male recipient of 21-4H spleen cells (group 2F), 18 and 34 wk after transfer, respectively; (H, solid trace) lethally irradiated (950 rad) 33-3 female recipient of nontransgenic F344 BM cells. Background fluorescence with an irrelevant antibody is shown in F, broken trace, and H, dashed trace.

Figure 5. Cell surface expression of HLA-B27 on CD4+ PBMC in recipients of BM or spleen cells. (A) Disease-affected lethally irradiated (1,050 rad) nontransgenic LEW male recipient of 21-4H BM cells (group 2D), 20 wk after transfer. (B) Disease-affected lethally irradiated (1,050 rad) nontransgenic F344 male recipient of 33-3 BM cells (group 3C), 13 wk after transfer. (C) Disease-affected lethally irradiated (1,050 rad) nontransgenic LEW male recipient of 21-4H spleen cells (group 2F), 34 wk after transfer (same rat as in Fig. 4 F, solid trace). (D) Healthy, lethally irradiated (1,050 rad) nontransgenic LEW male recipient of 21-4H spleen cells (group 2F), 34 wk after transfer (same rat as in Fig. 4 F, dashed trace). Dot plots were obtained for cells with mononuclear morphology, as defined by light scatter. Quadrants were defined by light scatter. Quadrants were defined by light scatter. Quadrants were defined by light scatter. Quadrants were defined by light scatter. Quadrants were defined by light scatter. Quadrants were defined by light scatter. Numbers in parenthesis refer to the CD4+ population displaying morphology and surface markers characteristic of monocytes.
prompt improvement. Skin lesions and diarrhea disappeared completely in all five recipients after 8 wk. Two of these later developed an unexplained cachexia that was fatal 14 and 18 wk after transfer, but the remaining three all survived at least 9 mo after transfer free of clinical disease. Flow cytometry showed almost complete absence of B27+ cells in the BM (Fig. 4 H), blood, and peripheral lymphoid organs (data not shown). Histological examination of the gut showed only mild evidence of inflammation and scarring of the colon in the group reconstituted with nontransgenic BM (Fig. 1 D), whereas the group receiving 33-3 BM showed typical severe lesions (data not shown). One of the two rats replaced with normal F344 BM that died spontaneously showed hepatic abnormalities consisting of fatty degeneration with a non-specific granulomatoid reaction, probably secondary to infection. The liver of the other rat was normal except for rare, mild periductal mononuclear cell infiltration that is also occasionally seen in the spontaneous disease (data not shown).

Discussion

Disease Transfer and the Requirement for Bone Marrow Engraftment. Gut, skin, and joint disease in the recipients was induced by transfer of BM cells from the disease-prone 21-4H and 33-3 lines but not from the disease-resistant 21-4L line or from nontransgenic donors. In view of the correlation between disease susceptibility and high levels of B27 expression (3), this suggests strongly that the disease susceptibility of the 21-4H and 33-3 lines can be attributed to expression of high levels of B27 in one or more populations of BM-derived cells. Although the results with whole BM cells did not exclude the possibility that transfer of disease required cotransfer of mature effector cells (14), subsequent experiments indicated that irradiated nontransgenic recipients of T cell-depleted BM (our unpublished findings) or FL cells from donors of disease-prone lines also developed inflamma-
tory disease similar to that occurring spontaneously in the donor lines. It thus appears that hematopoietic precursor cells from premorbid rats of the disease-prone lines are sufficient to confer disease susceptibility in an otherwise nontransgenic environment.

In contrast to these results with BM and FL cell transfer, comparable numbers of spleen or LN cells injected into similarly irradiated recipients either induced less severe pathologic manifestations, almost exclusively limited to late perifolliculitis in the case of sublethally irradiated recipients, or had no pathological effect. These observations predicted that transfer of disease, particularly gut and joint disease, requires engraftment of BM stem cells in the recipients, and this prediction was supported by immunofluorescence analysis of BM and peripheral lymphoid cells from the various recipients. Because spleen or LN cells in the absence of BM engraftment failed to transfer disease, the findings further suggest a necessary role played by BM-derived hematopoietic precursors for which cells from peripheral lymphoid organs such as mature lymphocytes cannot readily substitute. Because of their high levels of expression of HLA-B27 in the disease-prone transgenic lines and also because of their involvement in MHC-dependent immune responses, BM-derived APC from the monocyctic or dendritic cell lineages are likely to play such a role. Evidence for this idea was provided by the observation that the incidence and progression of disease in the recipients correlated with the accumulation of a donor-derived monocytic population. Recovery from disease observed in 33-3 rats after replacement with nontransgenic BM may have been linked to this same population of precursor cells, although this was not formally demonstrated. Although it is conceivable that donor cells were rejected by the nontransgenic recipients in proportion to the level of expression of B27, such that spleen or LN cells showed less efficiency in transferring disease than the more immature cells provided by BM or FL cells, this was unlikely to happen in the 21-4L recipients, and the long-term persistence of mature B27 donor lymphocytes regularly observed in the blood of nontransgenic recipients of 21-4H or 33-3 LN or spleen cells also argues against this possibility.

Although a BM-derived nonlymphocyte population appears to be necessary for disease transfer, it is likely that T lymphocytes are also essential to the disease pathogenesis, since athymic nude rats bearing the 33-3 transgene locus fail to develop inflammatory disease unless reconstituted with T cells, even though BM cells from these nude rats can transfer disease to irradiated F344 recipients (our unpublished results).

**Differences between the Transferred Disease and GVHD.** Like B27-associated disease, GVHD in the rat can cause inflammatory lesions of the gut, skin, and joints (15-17). It was therefore of concern, especially after genetic contamination of the 21-4H line was identified, that BM cells from these donors might have actually induced a form of GVHD that we took for passively transferred B27-associated disease. This seems unlikely for several reasons. First, the clinical and histologic appearance and the time course of the both the spontaneous and passively transferred disease in the B27 rats differ substantially from that described for either acute or chronic GVHD in the rat. For example, the prominent hepatic and salivary gland abnormalities, vasculitis and pulmonary fibrosis of GVHD, are not found in the B27-associated disease. Colonic lesions are lacking in chronic GVHD in the rat but are a consistent and prominent feature of B27-associated disease; and the scleroderma-like skin changes of chronic GVHD are not found in the B27-associated disease, the B27-associated skin lesions being instead hyperplastic. Second, reproducible disease transfer was obtained with both 21-4H and 33-3 donors, the latter of which showed no evidence of genetic contamination. Third, the nontransgenic parents of the 21-4H donors in later transfers were all authentic LEW males, and hence there was no immunologic basis for GVHD in the nontransgenic LEW recipients of cells from these donors. Fourth, LN cells, which are usually potent inducers of GVHD (18), were much less efficient in inducing disease manifestations than cells lacking mature T lymphocytes such as FL cells. Fifth, disease transfer with FL cells segregated perfectly with the B27 status of the 21-4H fetal donors (groups 2E and H), and independently of variations in genetic background (our unpublished observations). Therefore, despite the potential complication presented by the genetic contamination of the LEW lines, the data strongly suggest that the transferred disease is a B27 transgene-associated phenomenon, irrespective of the genetic background.

**Comparisons with other Disease Transfer Models.** The passive transfer of disease in the B27 transgenic rats bears similarity to studies of the BB/W rat and NOD mouse models of MHC-linked insulin-dependent diabetes mellitus, in which transfer of disease with BM cells (19-21), participation of T cells in disease pathogenesis (22, 23), and a lack of requirement for genetic abnormality in the target tissue (20) have been identified. BM cells have also been implicated in the transfer or treatment of other autoimmune disease in humans and in animal models (24, 25), and in the efficient transfer of the delayed hypersensitivity response in rats (26). Although T effector cells are known to participate in these processes, additional marrow-derived elements such as APC (27), also are thought to be involved.
Specificity of Disease for B27, Potential Mechanisms, and Relationship to Human Disease. The disease of the B27 transgenic rats bears a striking association to B27-associated disease in humans, the pathogenesis of which is still poorly understood. A variety of mechanisms have been proposed, largely based on the well documented triggering role of infectious agents in B27-associated reactive arthritis. These include molecular mimicry (28, 29), peptide binding (30), and altered bacterial handling (31, 32). Most hypotheses have depended upon the principle that class I MHC molecules are expressed in most somatic tissues (33). Since it is clear from the results presented here that B27 need not be expressed in nonmarrow-derived elements in the thymus, or in sites of disease such as intestinal mucosa, synovium, or skin in order for B27-mediated disease in rats to be induced in these sites, these results should help exclude consideration of hypotheses that would depend upon B27 expression in non-BM-derived tissues. However, the specific disease mechanism remains a matter of speculation. BM-derived APC expressing B27 at the high levels seen in the disease-susceptible lines could potentially modify immune regulation in the thymus, where BM-derived cells participate in negative and perhaps positive selection of maturing thymocytes (34, 35) and/or in peripheral lymphoid tissue, where presentation of antigen above a critical level or in a nonphysiologic manner (36) may trigger an immunopathologic process. B27 transgenic rats can mount strong B27-restricted CTL responses (37), but it is not yet known whether this is the predominant effector mechanism in either the spontaneous or transferred disease. Presentation of a B27-derived peptide (38, 39) by rat MHC molecules is another potential disease mechanism that has not yet been excluded. Further experiments should help identify the role of T cells, the specific BM-derived populations responsible for the transferred disease, and the molecular role of B27, as well as whether recognition of an exogenous antigen (31) or a self-antigen (40) is involved. The transfer model described here will be an essential element in these experiments.

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