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# Evidence for a Role of IL-17 in Organ Allograft Rejection: IL-17 Promotes the Functional Differentiation of Dendritic Cell Progenitors[1]

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IL-17 is a T cell-derived cytokine that stimulates stromal cells and macrophages to secrete proinflammatory cytokines. We hypothesized that IL-17 might play a role in alloimmune responses, and that interference with its activity might suppress allograft rejection. IL-17R:Fc or control IgG was added at the start of mouse MLR or was administered i.p. (100–500 $\mu$g/day) for different durations post-transplant to murine recipients of MHC-mismatched cardiac allografts. IL-17R:Fc (50–200 $\mu$g/ml) markedly inhibited T cell proliferation in vitro and significantly prolonged nonvascularized cardiac allograft median survival time from 13 to 20 days (100 $\mu$g/day; days 0 and 1) or to 19 days (100–300 $\mu$g/day; days 0–4). Survival of vascularized grafts was also extended significantly from 10.5 to 19 days by IL-17R:Fc (500 $\mu$g/day; days 0–6). To address a possible mechanism by which IL-17 may promote alloreactivity, we examined the influence of IL-17 on the differentiation and function of bone marrow-derived cells propagated in granulocyte-macrophage CSF with or without IL-4 to promote dendritic cell (DC) growth. A minor proportion of CD11c$^+$ DC expressed the IL-17R. IL-17 promoted the maturation of DC progenitors, as evidenced by increased cell surface expression of CD11c, costimulatory molecules (CD40, CD80, CD86), and MHC class II Ag, and allostimulatory capacity. IL-17 had a lesser effect on the phenotype and function of more fully differentiated myeloid DC. These findings suggest a role for IL-17 in allogeneic T cell proliferation that may be mediated in part via a maturation-inducing effect on DC. IL-17 appears to be a novel target for therapeutic intervention in allograft rejection.   *The Journal of Immunology,* 1999, 162: 577–584.

Interleukin-17 is a T cell-derived cytokine identified originally by Rouvier et al. (1) as CTL-associated Ag-8 (CTLA-8).[3] The mouse (m) CTLA-8 gene has been cloned, and its complete nucleotide sequence has been determined (2). There is striking homology between CTLA-8 and the protein encoded by the open reading frame 13 of *Herpesvirus saimiri* (57% homology over 147 amino acids (aa)) (3, 4). Human (hu) IL-17, a glycoprotein of 155 aa secreted primarily by activated CD4$^+$ T cells (5), exhibits an overall sequence identity of 72% at the aa level with open reading frame 13 of *H. saimiri* and 63% with mIL-17 (6, 7). The huIL-17R gene has been localized to chromosome 22 and has no sequence similarity with any other known cytokine receptor family (8). Northern analysis has revealed mIL-17R expression in virtually all cells and tissues (4, 5). This ubiquitous distribution of the IL-17R contrasts with the restricted expression of IL-17 to T cells (1, 4–6, 9).

IL-17 induces activation of the transcription factor NF-$\kappa$B in a variety of cell types (9) and stimulates stromal elements, such as fibroblasts, endothelial cells, and epithelial cells to secrete IL-6, IL-8, granulocyte CSF (G-CSF), and PGE$_2$, a process that can be blocked by anti-IL-17 mAb. In addition, IL-17 induces the secretion of IL-1$\beta$ and TNF-$\alpha$ by human macrophages (10) and enhances IL-1-induced IL-6 and leukemia inhibitory factor production by rheumatoid synoviocytes (11). Besides inducing proinflammatory cytokine production, IL-17 exhibits indirect hemopoietic activity by enhancing the ability of fibroblasts to sustain the growth of CD34$^+$ hemopoietic progenitors and directing their maturation into neutrophils (9). IL-17 also augments mature T cell proliferation induced by suboptimal concentrations of PHA. Recently, an IL-17 antagonist, soluble mIL-17R:Fc fusion protein, has been found to inhibit IL-2 production and T cell proliferation induced by PHA, Con A, anti-TCR mAb, or anti-CD28 mAb, suggesting a key role for endogenously produced IL-17 in T cell growth (4, 5).

We hypothesized that IL-17 might play a role in the generation of alloimmune responses, and we have tested the influence of mIL-17R:Fc on alloimmune reactivity, both in vitro and in vivo. In view of the capacity of IL-17 to exhibit hemopoietic activity and to further explore its immunologic role, we also examined the influence of IL-17 on the in vitro differentiation and function of bone marrow-derived dendritic cells (DC), potentially the most potent donor-derived APC for T cell activation and proliferation (12). The results reveal effects of IL-17 on the maturation of DC progenitors, allogeneic T cell proliferation, and the expression of alloimmune reactivity. They also suggest that IL-17 plays a role in organ allograft rejection, and that IL-17 antagonism may have potential for antirejection therapy, either alone or in combination with other immunosuppressive agents exhibiting complementary modes of action.

[3] Abbreviations used in this paper: CTLA-8, cytotoxic T lymphocyte-associated Ag-8; m, mouse; aa, amino acids; hu, human; G-CSF, granulocyte CSF; DC, dendritic cell; rm, recombinant mouse; rhu, recombinant human; GM-CSF, granulocyte-macrophage CSF; MST, median survival time.

## Materials and Methods

### Animals

Male C57BL/10J (B10; H2[b]) and C3H (H2[k]) mice, 8–10 wk of age, were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed in the Central Animal Facility of the University of Pittsburgh Medical Center. BALB/c (H2[d]) and C57BL/6 (B6; H2[b]) mice were obtained from Taconic Farms (Germantown, NY) and were bred and housed at Immunex Corp. (Seattle, WA). All animals were maintained under specific pathogen-free conditions.

### Recombinant mIL-17R:Fc

A soluble rmIL-17R:Fc fusion protein constructed by fusing the extracellular domain (residues 1–323) of IL-17R to the Fc portion of huIgG1 (4) was reconstituted in sterile HBSS (Life Technologies, Grand Island, NY). For in vivo use, it was injected i.p. Controls received human or rat IgG (Sigma, St. Louis, MO).

### Recombinant huIL-17 and mIL-17R mAb

Recombinant huIL-17 was purchased from R&D Systems (Minneapolis, MN). Purified rat IgG mAb against the mIL-17R (M177) was provided by Immunex Corp.

### Nonvascularized heart transplantation

Neonatal (day 1) B6 hearts were transplanted into the ear pinnae of adult BALB/c recipients, using the method of Fulmer et al. (13), with modifications as previously described (14). Following transplantation, the mice were assigned to four different groups, with four animals in each group. Groups 1 and 2 received IL-17R:Fc or huIgG at 100 μg/day i.p. on days 0–2, where day 0 was the day of transplant. Groups 3 and 4 received rmIL-17R:Fc or rat IgG at 100 μg/day i.p. on days 0–3 post-transplant. The heart grafts were monitored for contractile activity on a daily basis by a blinded observer using a stereomicroscope. Rejection was determined as cessation of heartbeat.

### Vascularized heart transplantation

Heterotopic heart transplantation was performed in the B10 to C3H combination (MHC class I, class II, and multiple non-MHC Ag disparities) using techniques adapted from the rat procedure of Ono and Lindsey (15). The heart was transplanted into the abdomen with end-to-end anastomosis of aorta to aorta and pulmonary artery to vena cava. Immediately following transplantation, the animals were treated with varying doses of IL-17R:Fc (100–500 μg/day) for different periods of time, and graft survival was evaluated. Control mice received no treatment. Rejection was defined by the cessation of cardiac contraction after daily palpation through the abdominal wall.

### Mixed leukocyte reactions

Either bulk splenocytes or spleen cells that were highly T cell enriched by passing them through a nylon wool column at 37°C in 5% CO2 in air for 30 min were set up (2 × 10^5 cells/well) as responders in 96-well microtiter plates (Corning, Corning, NY). They were cocultured with graded concentrations of gamma-irradiated (20 Gy) stimulator cells in RPMI 1640 (Life Technologies) complete medium, containing 10% (v/v) heat-inactivated FBS (Life Technologies), 2 mM L-glutamine, 50 U/ml penicillin and streptomycin, and 2 mM nonessential aa for 3–4 days at 37°C in 5% CO2 in air. Sixteen to eighteen hours before the end of the culture period, individual wells were pulse labeled with 1 μCi of [3H]thymidine. The plates were harvested, and the amount of radioisotope incorporated into the cells was determined using a beta scintillation counter. Results are expressed as the mean counts per minute ± 1 SD and are representative of experiments performed at least twice.

### Propagation of DC progenitors from bone marrow

B10 mouse bone marrow cells were isolated and DC progenitors were propagated using the procedure described initially by Inaba et al. (16), with modifications as previously described (17). Briefly, 2 × 10^6 bone marrow cells were cultured in 24-well plates in 2 ml of RPMI 1640 complete medium containing various concentrations of rmGM-CSF (1, 2, or 4 ng/ml) with or without rm IL-4 (1000 U/ml; cytokines from Schering-Plough Research Institute, Kenilworth, NJ). On day 2 of culture, the medium was gently removed and spun down, and 1 ml of this old medium along with 1 ml of fresh medium containing cytokines were added to the culture. This step allowed the depletion of nonadherent granulocytes without dislodging clusters of DC progenitors that were loosely attached to a monolayer of
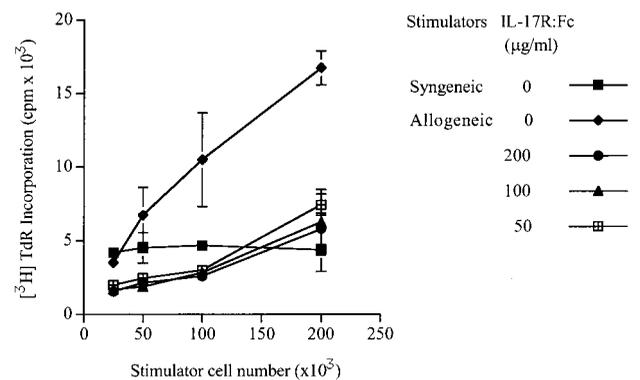


**FIGURE 1.** Inhibitory effect of IL-17R:Fc on proliferative responses of mouse T lymphocytes in primary MLR. Varying numbers of gamma-irradiated B10 (H-2[b]) or C3H (H-2[k]) bulk spleen stimulator cells were cultured with C3H spleen cells for 4 days. Various concentrations of IL-17R:Fc (50–200 μg/ml) or huIgG (isotype control, 100 μg/ml) were added at the start of the cultures. [3H]TdR was added for the last 18 h. Results are mean ± 1 SD from triplicate cultures and are representative of two separate experiments.

plastic-adhered macrophages. On day 5, floating cells (many of which exhibited typical DC morphology) were harvested and incubated overnight (18 h) in medium containing either no cytokine or various concentrations of rhuIL-17. At the end of the culture period, the cells were harvested, and phenotypic analyses were performed. The functional capacity of the cells was tested by adding them as stimulators to either C3H spleen cells or purified T cell responders in a one-way MLR.

### Staining for cell surface Ags

The surface phenotype of the bone marrow-derived cells was analyzed after gating for DC by cytofluorography, using an EPICS Elite flow cytometer (Coulter, Hialeah, FL). Before staining with the relevant mAb, the cells were incubated with 10% (v/v) normal goat serum (Vector Laboratories, Burlingame, CA) in HBSS at 4°C for 30 min to eliminate nonspecific binding. The cells were then washed once in HBSS containing 0.1% (v/v) BSA (Sigma) and analyzed using FITC-labeled Abs (PharMingen, San Diego, CA) for expression of the murine DC-restricted marker CD11c, costimulatory molecules (CD40, CD80, and CD86), and MHC class II Ag (I-A[b]). Ig isotype controls were included for each mAb. After staining, the cells were fixed in 1% (v/v) paraformaldehyde in saline before analysis. Five thousand events were accumulated for each sample. In the same experiments, two-color staining for CD11c and other Ags of interest was performed using phycoerythrin- and FITC-labeled mAbs, respectively. Data are presented as percentages of positive cells, after deduction of appropriate isotype control values.

### Statistics

The significances of differences were determined using the nonparametric Wilcoxon rank-sum test (graft survival data) or Student's t test, as appropriate.

## Results

### IL-17R:Fc inhibits anti-donor T cell proliferative responses in vitro

To test for a role for IL-17 in alloimmune responses in vitro, variable numbers of gamma-irradiated normal allogeneic (B10) stimulator splenocytes were added to a fixed number of C3H bulk spleen cells together with varying doses of rmIL-17R:Fc (50–200 μg/ml) and cultured for 4 days. As shown in Fig. 1, each dose of the IL-17 antagonist tested markedly inhibited T cell proliferation compared with Ig isotype-treated controls. These findings suggest a role for IL-17 in the generation of allogeneic T cell responses and indicate the immunosuppressive potential of IL-17R:Fc.

### IL-17R:Fc prolongs nonvascularized cardiac allograft survival

B6 recipients of day 1 BALB/c heart grafts injected with 100 μg/day rmIL-17R:Fc on days 0–2 post-transplant exhibited significantly prolonged graft survival compared with control animals that

Table I.  *mIL-17R:Fc prolongs MHC-mismatched nonvascularized heart allograft survival[a]*

| Treatment, μg/day (timing) | Graft Survival Time (days) | Mean Survival Time (days) | Median Survival Time (days) |
|---|---|---|---|
| huIgG 100 (days 0–2) | 13, 13, 13, 15 | 13.5 ± 0.5 | 13 |
| mIL-17R:Fc 100 (days 0–2) | 20, 20, 20, 20 | 20* | 20 |
| Rat IgG 300 (days 0–3) | 11, 14, 14, 14 | 13.3 ± 0.8 | 14 |
| mIL-17R:Fc 100 (days 0–3) | 19, 19, 19, 21 | 19.5 ± 0.5[†] | 19 |

[a] The strain combination was BALB/c (H2[d]) to B6 (H2[b]).
* $p < 0.001$ when compared to control group given huIgG.
[†] $p < 0.003$ when compared to control group given rat IgG.

received hu IgG (Table I). Graft median survival time (MST) was prolonged from 13 to 20 days ($p < 0.005$). Similarly, B6 recipients treated with the same dose of fusion protein from days 0–3 post-transplant exhibited prolonged graft survival compared with controls. However, increasing the duration of treatment from 2 to 4 days post-transplant did not further extend graft survival time. The increase in MST observed following administration of IL-17R:Fc strongly implicates endogenous IL-17 in the generation and/or expression of anti-donor immune reactivity.

### IL-17R:Fc prolongs vascularized cardiac allograft survival

We next evaluated the influence of rmIL-17R:Fc on vascularized heart allograft survival. Different strains were used from those used for the nonvascularized grafts, to test whether the therapeutic effect could be observed in another MHC-disparate combination. Administration of rmIL-17R:Fc (500 μg/day) to C3H recipients of B10 cardiac allografts on days 0–6 post-transplant resulted in prolonged graft MST from 10.5 to 19 days ($p < 0.001$) (Table II). A lower dose of the fusion protein (200 μg/day) administered for varying periods post-transplant did not significantly affect graft survival. Overall, these findings using two experimental models indicate that by blocking the effects of IL-17, IL-17R:Fc can inhibit organ allograft rejection.

### IL-17 promotes the phenotypic development of DC progenitors

Bone marrow-derived DC are crucial APC for the activation of naive T cells (12). In normal heart and other nonlymphoid tissues, the interstitial DC are thought to be at an immature stage of phenotypic and functional development (18–20). After organ transplantation, these cells migrate to lymphoid tissue (17, 21, 22) where, as mature interstitial DC in T-dependent areas, they instigate allogeneic T cell activation and proliferation (20, 21). To determine whether IL-17 might influence the maturation of DC progenitors, rhuIL-17 was added to cultures of GM-CSF-stimulated bone marrow-derived cells, and its influence on their phenotype and function was determined. Cells propagated in 4 ng/ml

GM-CSF for 5 days, as described in *Materials and Methods*, were exposed to IL-17 (10 or 20 ng/ml) in the absence of other exogenous cytokines for 18 h. They were then analyzed for the expression of various cell surface markers by single color flow cytometry. As shown in Fig. 2, IL-17 treatment increased the intensity of staining for CD11c, CD40, and MHC class II (I-A[b]) and, more strikingly, up-regulated CD80 and CD86 expression. This maturation-promoting effect of IL-17 was observed consistently in five separate experiments. It was also observed when DC progenitors were propagated in a reduced concentration of GM-CSF (1 or 2 ng/ml) and 1000 U/ml IL-4, and then exposed to IL-17 under conditions identical with those described above (Fig. 3).

### IL-17 has a lesser effect on the phenotype of more fully differentiated DC

To ascertain whether IL-17 might affect more fully differentiated bone marrow-derived DC, 5-day cultures stimulated with 4 ng/ml GM-CSF and 1000 U/ml IL-4 and expressing comparatively high levels of CD11c, costimulatory molecules, and MHC class II were treated with IL-17 for 18 h. They were then analyzed by flow cytometry. In contrast to the effects on the immature cell population, the expression of key cell surface functional molecules was not significantly affected by exposure to IL-17 (Fig. 4).

### IL-17 up-regulates the expression of costimulatory molecules on CD11c^{+} cells

To confirm that the changes in cell surface phenotype induced by IL-17 occurred on DC, two-color flow analysis of CD80, CD86, or MHC class II and the DC-restricted Ag CD11c was performed. Cells propagated in various concentrations of GM-CSF (1, 2, or 4 ng/ml) in the absence or the presence of IL-4 (1000 U/ml) for 5 days, then exposed overnight (18 h) to IL-17 (20 ng/ml), were harvested, stained, gated for DC, and analyzed. As shown in Table III, IL-17 up-regulated the expression of CD80 and CD86 on CD11c^{+} cells at all concentrations of GM-CSF tested (with or without IL-4). Up-regulation of IA[b] (MHC class II) expression, however, was only evident at comparatively low concentrations of GM-CSF.

### Expression of IL-17R on CD11c^{+} cells

Since responsiveness of DC to IL-17 may be dependent on cell surface expression of the IL-17R, 5-day cultures of either GM-CSF-stimulated or GM-CSF- plus IL-4-stimulated cells were analyzed by two-color flow staining for coexpression of CD11c and IL-17R. As shown in the results of a representative experiment (Fig. 5A), 18% of the GM-CSF-stimulated CD11c^{+} cells were IL-17R^{+}; culture in GM-CSF and IL-4 resulted in a lower incidence of CD11c^{+} DC expressing surface IL-17R (8%). Also shown in Fig. 5, *B* and *C*, are the results of staining for IL-17R on normal, resting, and Con A-activated bulk splenocytes and T cells.

Table II.  *mIL-17R:Fc prolongs MHC mis-matched vascularized heart allograft survival[a]*

| Dosage, μg/day (timing) | Graft Survival Time (days) | Mean Survival Time (days) | Median Survival Time (days) |
|---|---|---|---|
| None | 8, 9, 10, 10, 11, 12, 12, 13 | 10.6 ± 1.7 | 10.5 |
| 200 (days 0–3) | 7, 9, 11, 13 | 10.0 ± 2.6 | 10 |
| 200 (days 0–6) | 10, 12, 12, 13, 17, 21, 22, 35 | 17.8 ± 8.2 | 15 |
| 500 (days 0–6) | 14, 14, 16, 19, 19, 22, 23, 36, 48 | 23.4 ± 11.4 | 19* |

[a] The strain combination was B10 (H2[b]) to C3H (H2[k]).
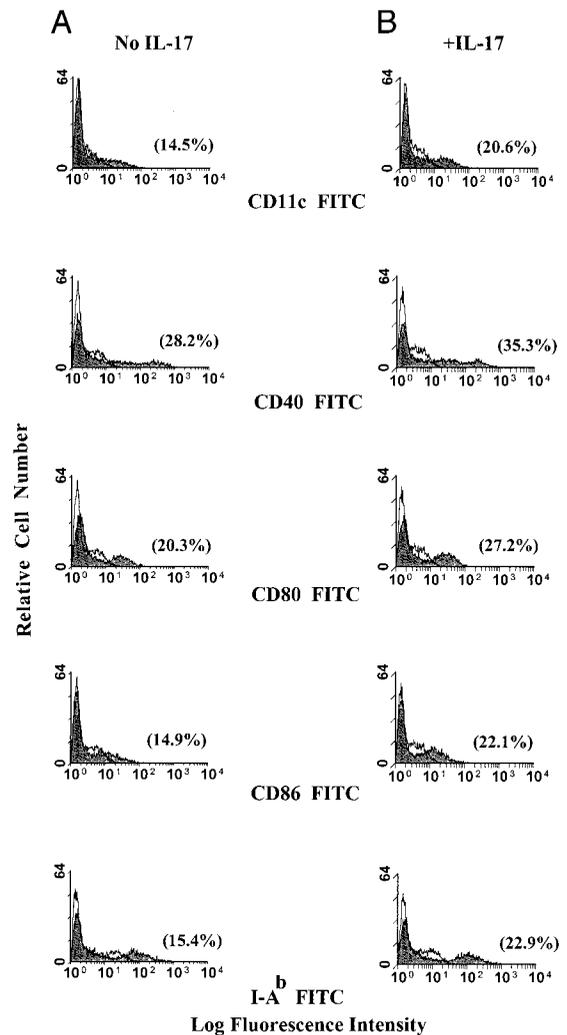* $p < 0.0001$ when compared to the control group.
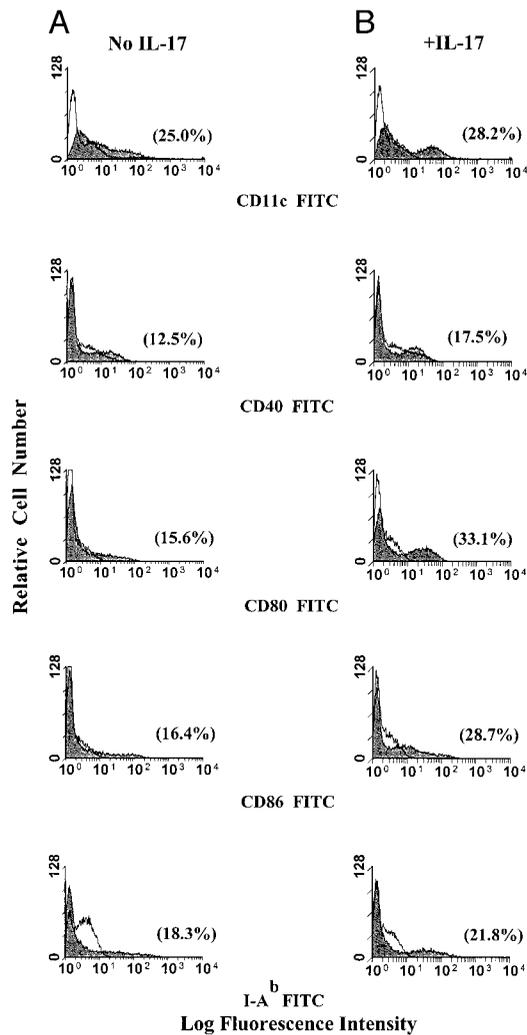
**FIGURE 2.** IL-17 promotes the phenotypic differentiation of DC progenitors in bone marrow (BM) cell cultures stimulated with GM-CSF (4 ng/ml) alone. Freshly isolated B10 BM cells were cultured to enrich for DC progenitors, as described in *Materials and Methods*. Cultures were maintained for 5 days, then the cells were exposed to IL-17 (10 ng/ml) or to no exogenous cytokine for 18 h before harvesting. Following staining with FITC-conjugated mAbs directed against the DC-restricted Ag CD11c, costimulatory molecules (CD40, CD80, CD86), and MHC class II (I-A$^b$) Ag, the cells were analyzed by flow cytometric analysis. *A*, No IL-17 treatment; *B*, IL-17 treated. Open profiles denote isotype controls. The experiment was conducted five times, and the results shown are from a single representative experiment.

**FIGURE 3.** IL-17 promotes the phenotypic differentiation of DC progenitors in bone marrow (BM) cell cultures stimulated with GM-CSF (2 ng/ml) and IL-4 (1000 U/ml). Freshly isolated B10 BM cells were cultured to enrich for DC progenitors, as described in *Materials and Methods*. Cultures were maintained for 5 days, then the cells were exposed to IL-17 (10 ng/ml) or to no exogenous cytokine for 18 h before harvesting. Following staining with FITC-conjugated mAbs directed against the DC-restricted Ag CD11c, costimulatory molecules (CD40, CD80, and CD86), and MHC class II (I-A$^b$) Ag, the cells were analyzed by flow cytometric analysis. *A*, No IL-17 treatment; *B*, IL-17 treated. Open profiles denote isotype controls. The experiment was conducted three times, and the results shown are from a single representative experiment.

The incidence of positive cells was increased following their activation, suggesting that activated T cells in culture may be responsive to IL-17.

*IL-17 promotes T cell proliferation induced by DC progenitors*

The influence of exposure to IL-17 on the allostimulatory capacity of cultured DC progenitors was determined by adding various concentrations of rhuIL-17 (10–40 ng/ml) to bulk splenocytes at the start of primary (3-day) MLR induced by 5-day GM-CSF (4 ng/ml)-stimulated cells. As shown in Fig. 6 and consistent with its influence on the phenotypic maturation of these comparatively immature cells (Fig. 2), IL-17 increased the T cell proliferative response induced by DC progenitors in a dose-related manner. These data are consistent with a stimulatory

action of IL-17 on allogeneic T cell responses mediated at least in part by promotion of the functional maturation of bone marrow-derived APC (DC).

*Pre-exposure of DC progenitors to IL-17 augments their allostimulatory activity*

To exclude the possibility that IL-17 may augment T cell proliferative responses by a direct action on T cells rather than by a maturation-inducing effect on DC progenitors, we investigated the allostimulatory properties of DC progenitors pre-exposed to IL-17. Cells propagated in GM-CSF (1 ng/ml) for 5 days and then either left untreated or exposed to IL-17 (20 ng/ml) for an additional 18 h were used as stimulators of purified T cells in 3-day MLR. Co-cultures of IL-17-pre-exposed DC progenitors and allogeneic T
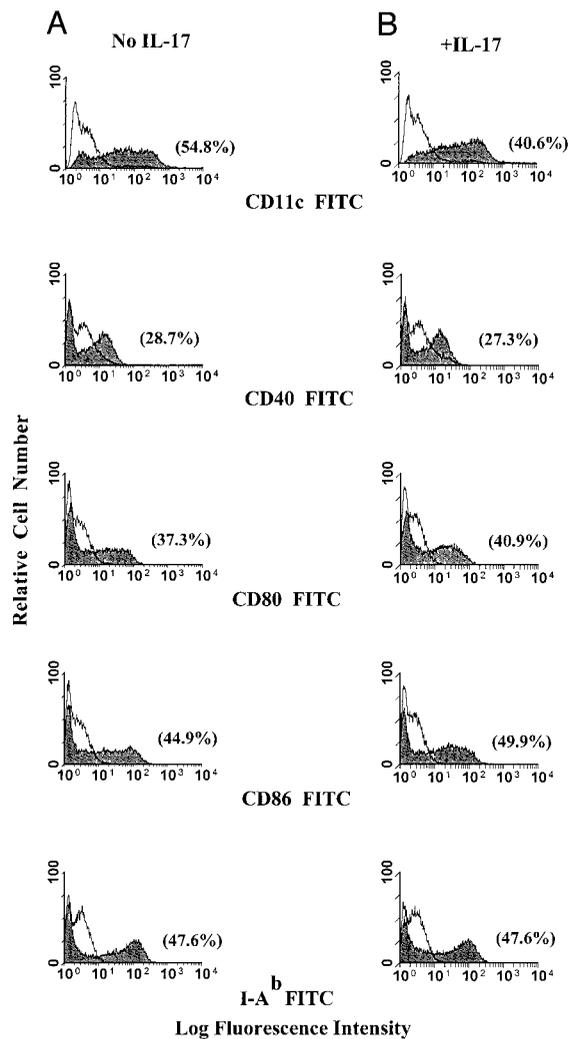
**FIGURE 4.** Exposure to IL-17 does not affect costimulatory molecule or MHC class II Ag expression on more fully differentiated DC. B10 BM-derived cells were cultured for 5 days in GM-CSF (4 ng/ml) and IL-4 (1000 U/ml), as described in *Materials and Methods*. The cells then remained untreated or were exposed for 18 h to IL-17 (10 ng/ml) or to no exogenous cytokine. The cell surface phenotype of the cells was then examined by mAb staining and flow cytometric analysis. *A*, No IL-17 treatment; *B*, IL-17 treated. Open profiles denote isotype controls. The experiment was repeated five times, and the results shown are from a single representative experiment.

**Table III.** *Exposure to IL-17 upregulates the expression of CD80, CD86 and MHC class II on CD11c⁺ DCᵃ*

| Phenotype | IL-17 | GM-CSF (ng/ml): % Positive Cells | | |
|---|---|---|---|---|
| | | 1 | 2 | 4 |
| CD11c⁺ | − | 18.0 | 36.2 | 47.2 |
| | + | 30.1 | 38.6 | 58.2 |
| CD11c⁺CD80⁺ | − | 7.1 | 14.7 | 21.2 |
| | + | 9.9 | 20.7 | 27.6 |
| CD11c⁺ CD86⁺ | − | 6.4 | 14.6 | 16.6 |
| | + | 11.4 | 19.1 | 21.9 |
| CD11c⁺IAᵇ⁺ | − | 10.2 | 23.4 | 38.4 |
| | + | 14.3 | 26.7 | 38.3 |
| | | GM-CSF (ng/ml) + IL-4 (1000 U/ml): % Positive Cells | | |
| CD11c⁺ | − | 43.3 | 51.4 | 65.1 |
| | + | 48.6 | 53.0 | 60.2 |
| CD11c⁺CD80⁺ | − | 9.2 | 12.6 | 22.1 |
| | + | 19.9 | 25.8 | 28.6 |
| CD11c⁺CD86⁺ | − | 16.5 | 21.0 | 29.9 |
| | + | 23.7 | 32.6 | 37.0 |
| CD11c⁺IAᵇ⁺ | − | 22.7 | 31.5 | 46.5 |
| | + | 20.8 | 30.3 | 36.6 |

ᵃ Bone marrow-derived cells propagated in GM-CSF or GM-CSF + IL-4 for 5 days, as described in *Materials and Methods*, were exposed to rhuIL-17 for 18 h, then analyzed by two-color staining for coexpression of CD11c and either costimulatory (B7) molecules (CD80; CD86) or MHC class II (IAᵇ). The data are representative of three separate experiments.

## Discussion

Numerous cytokines are believed to play key roles in the processes of acute and chronic allograft rejection (23, 24). The cloning of these growth factors and their receptors has greatly expanded knowledge of their structure, function, and interaction, while blocking of their activity (e.g., using mAbs or soluble receptors) is, in many instances, a rational approach to the immunosuppressive therapy of allo- or autoimmunity (14, 25–31). IL-17, a T cell-derived cytokine-inducing cytokine (32), is known to stimulate stromal cells to secrete IL-6, IL-8, and the hemopoietic growth factor, G-CSF (4). It stimulates the production of inflammatory cytokines by macrophages (10) and induces ICAM-1 expression on fibroblasts (4). These inductive effects can be inhibited or blocked by specific anti-IL-17 mAb or by anti-inflammatory cytokines (4, 10).
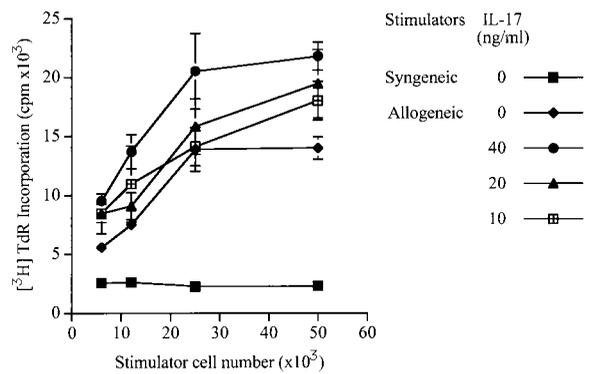
IL-17 also acts in concert with other cytokines to induce proinflammatory responses in various cell types. Both TNF-α and IFN-γ synergize with IL-17 to augment IL-17-induced secretion of IL-6 by rheumatoid synoviocytes (4, 5). Lotz et al. (33) have found IL-17 to promote synovial inflammation and cartilage degradation. Thus, IL-17 stimulated the expression of various genes (inducible cyclo-oxygenase, inducible nitric oxide synthase, IL-6, stromelysin, and collagenase) in normal human chondrocytes and augmented IL-1, IL-6, macrophage chemotactic protein-1, and inducible cyclo-oxygenase gene expression in synoviocytes. In addition, the joint tissues of patients with rheumatoid arthritis, psoriatic arthritis, or osteoarthritis revealed message for this cytokine (33). Yao et al. (5) have reported that together with TNF-α, IL-17 induces GM-CSF production by synovial fibroblasts. This functional interrelationship between IL-17 and numerous other cytokines (IL-1, IL-6, IL-8, macrophage chemotactic protein-1, GM-CSF, TNF-α, and IFN-γ) suggests a role for IL-17 in the induction and/or expression of alloimmune reactivity. Moreover, previous studies have shown that IL-17 augments T cell proliferation induced by a suboptimal mitogenic costimulus (PHA). In addition,

cells were also set up, with addition of IL-17 at the start of the MLR. Purified T cells were used as responders. As shown in Fig. 7A, pre-exposure of DC progenitors grown in a low concentration of GM-CSF (1 ng/ml) to IL-17 significantly augmented their allostimulatory activity (by approximately twofold) at all DC:T cell ratios tested (p < 0.05). This effect was more marked, although not consistently so over all DC:T cell ratios, when additional IL-17 (20 ng/ml) was added at the start of the MLR. When more fully differentiated DC (grown in 1 ng/ml GM-CSF and 1000 U/ml IL-4) were pre-exposed to IL-17 and used as stimulators, no significant effect of IL-17 pretreatment on their potent T cell stimulatory response was observed (Fig. 7B). Of interest was that further exposure of these highly stimulatory APC to IL-17 during the MLR tended to reduce their effect on T cell proliferation (p < 0.02 compared with untreated DC at the maximal response), suggesting a feedback regulatory mechanism.

**FIGURE 5.** *A*, Expression of IL-17R on CD11c⁺ DC. Cells were propagated in GM-CSF and IL-4 for 5 days, as described in *Materials and Methods*. They were stained with phycoerythrin-conjugated CD11c and anti-IL-17R- and FITC-conjugated mouse anti-rat IgG, and the incidence of CD11c⁺ IL-17R⁺ cells was determined by flow cytometric analysis. For comparative purposes, the expressions of IL-17R on resting and 48-h Con A-activated bulk spleen cells (*B*) and on resting and Con A-activated T cells (*C*) are shown. The data are representative of three separate experiments.



**FIGURE 6.** IL-17 promotes the allostimulatory activity of bone marrow (BM)-derived DC progenitors for bulk naive spleen cells. Freshly isolated B10 BM cells were cultured in GM-CSF (4 ng/ml) alone, as described in *Materials and Methods*, for 5 days, and then used as stimulators of C3H splenocytes in primary, 3-day MLR. Various concentrations of rhuIL-17 (10–40 ng/ml) were added at the start of cultures. The cultures were maintained for 72 h; [³H]TdR was added 18 h before cell harvest. Results are the mean ± 1 SD and are representative of three separate experiments.

the IL-17 antagonist IL-17R:Fc can inhibit IL-2 production and T cell proliferation induced by phytomitogens or anti-TCR or anti-CD28 mAbs (4). These observations suggest a role for endogenously produced IL-17 in the induction of proinflammatory cytokine release and T cell proliferation, with implications for effects on immune reactivity.

The present study shows for the first time that blocking the effects of IL-17 using IL-17R:Fc not only inhibits proliferative responses of T cells to alloantigens in vitro, but also significantly prolongs MHC-mismatched nonvascularized and vascularized cardiac allograft survival. In the nonvascularized heart transplant model, treatment of the recipients with 100 μg/day of fusion protein for only 3 days led to a significant increase in graft MST compared with that in control (huIgG-treated) animals. In the vascularized model, however, a larger dose of IL-17R:Fc (500 μg/day for 7 days post-transplant) was required to significantly prolong heart graft survival. This difference may have been due both to the use of different strain combinations in the two models and to the

apparent greater immunogenicity of the vascularized heart grafts, which survived for a shorter time than the nonvascularized grafts in unmodified hosts. Thus, higher levels of IL-17 as well as other cytokines may have been involved in the immune responses generated to the vascularized allografts, and hence, a higher dose of the fusion protein was required to achieve a protective effect. In vitro, the ability of IL-17R:Fc to inhibit allogeneic T cell proliferative responses further indicates a role for IL-17 in T cell activation and/or growth. The finding that IL-17R:Fc can enhance organ allograft survival as well as suppress allogeneic T cell alloresponses in vitro suggests that IL-17 is a proinflammatory cytokine with significant effects in the process of organ allograft rejection.

A number of different mechanisms have been proposed by which cytokines mediate graft destruction. IFN-γ and TNF-β are directly cytotoxic to the graft, IL-2 and IL-4 promote the expansion and differentiation of mature cytotoxic T cells, whereas IL-4, IL-5, and IL-6 promote B cell maturation and the development of specific, anti-graft alloantibodies (23, 24, 34, 35). Rejection is characterized by the infiltration of lymphocytes into the graft. While the CD4⁺ subset elaborates cytokines, CD8⁺ cells are primarily cytotoxic effector cells. It is reasonable to propose that IL-17 is elaborated by graft-infiltrating T lymphocytes, and that it plays a role (either directly or indirectly) in intensifying the local inflammatory response by acting on various cell types, such as T cells and fibroblasts, and by recruiting additional cells, including APCs, into the graft site. This may be achieved by inducing cytokines such as IL-8 and GM-CSF and by up-regulation of adhesion molecule expression. A subset of such recruited cells is likely to be DC progenitors, with the important characteristics of motility and migratory capacity (12, 20), enabling these cells to move from peripheral tissues (including the allograft) to regional lymphoid organs (21, 22). It is thus of interest that combination of IL-17 with TNF-α is effective in inducing the release of GM-CSF (9), a key growth- and maturation-inducing factor for myeloid DC (16).

Bone marrow-derived DC present in organ allografts, such as heart or liver, are believed to be in an immature state of development (18, 19) and to be influenced by various factors (such as Ags and the cytokine milieu) to migrate and undergo maturation. The present finding that IL-17 can serve to promote the phenotypic and functional maturation of DC progenitors suggests a mechanism by

A. GM-CSF

B. GM-CSF + IL-4
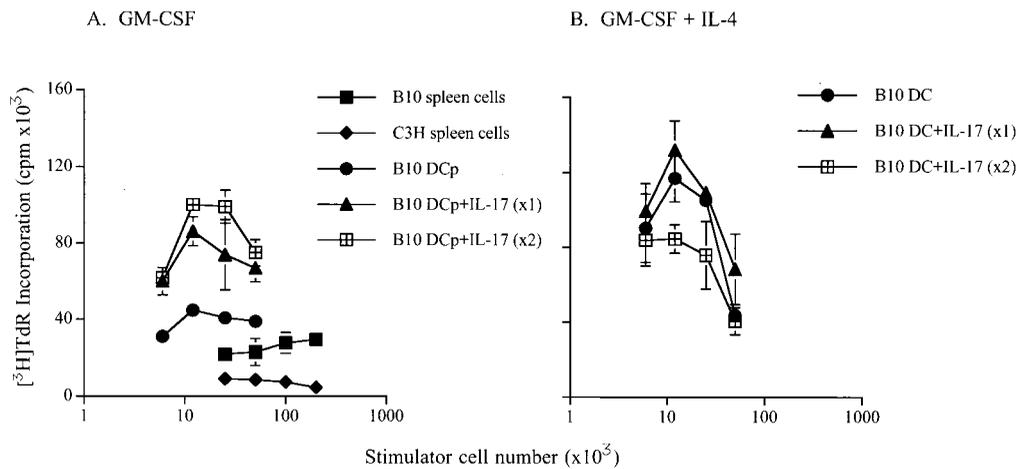


**FIGURE 7.** Pre-exposure of DC progenitors, but not more mature DC, to IL-17 augments their capacity to induce allogeneic T cell proliferation. DC progenitors (DCp; *A*) or more mature DC (*B*) were propagated in GM-CSF (1 ng/ml) or GM-CSF (1 ng/ml) plus IL-4 (1000 U/ml), respectively, for 5 days, as described in *Materials and Methods*. They were then exposed to either IL-17 (x1; 20 ng/ml) or to no exogenous cytokine for 18 h before testing in a 3-day primary MLR with highly purified C3H T cells as responders. [$^3$H]TdR was added for the last 18 h of culture. Cultures were also established to which additional IL-17 (x2; 20 ng/ml) was added at the start of the MLR. While additional IL-17 further increased the response of T cells to IL-17-pre-exposed DCp (*A*), some reduction in the stimulatory capacity of similarly treated mature DC was observed (*B*). The data are the mean ± 1 SD and are representative of three separate experiments.

which this cytokine may promote host T cell sensitization (allostimulation) and consequent graft rejection. Whether these effects on DC progenitors (and possibly other APC) may be mediated directly or indirectly is at present uncertain. We observed that approximately 20% of bone marrow-derived DC progenitors expressed IL-17R, as determined by flow cytometric analysis, and that expression of the receptor appeared to be reduced on more mature DC. Thus, it is likely that in the present studies, only a minor population of DC progenitors was directly responsive to IL-17. Since IL-17 is known to promote the secretion of a variety of cytokines, including hemopoietic growth factors from stromal cells and macrophages, its stimulatory effects on DC progenitors in vitro and its presumed influence on these cells in vivo may be mediated in part by an indirect action on stromal cell elements, such as endothelial cells and fibroblasts. The relative inability of more fully differentiated, highly stimulating DC to respond to IL-17 and its capacity to exert a negative effect on T cell proliferation when added at the start of vigorous MLRs may reflect the refractoriness of potent APC and/or T cells to the cytokine and perhaps feedback inhibition mediated via IL-17-IL-17R interaction.

Taken together, the findings clearly indicate the effects of the IL-17/IL-17R pathway on alloimmune responses. The capacity of the IL-17R:Fc fusion protein to prolong organ allograft survival suggests that IL-17 antagonism may have potential for therapy of transplant rejection and perhaps other immune-mediated disorders, possibly in conjunction with immunosuppressive agents with complementary modes of action.

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