THE JOURNAL OF IMMUNOLOGY

IL-5 Mediates Eosinophilic Rejection of MHC Class II-Disparate Skin Allografts in Mice

This information is current as of May 8, 2014.

Alain Le Moine, Murielle Surquin, François Xavier Demoor, Jean Christophe Noël, Marie-Anne Nahori, Marina Pretolani, Véronique Flamand, Michel Y. Braun, Michel Goldman and Daniel Abramowicz

References	This article **cites 44 articles**, 19 of which you can access for free at: http://www.jimmunol.org/content/163/7/3778.full#ref-list-1
Subscriptions	Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscriptions
Permissions	Submit copyright permission requests at: http://www.aai.org/ji/copyright.html
Email Alerts	Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/cgi/alerts/etoc

IL-5 Mediates Eosinophilic Rejection of MHC Class II-Disparate Skin Allografts in Mice[1]

Alain Le Moine,*† Murielle Surquin,*† François Xavier Demoor,* Jean Christophe Noël,‡ Marie-Anne Nahori,§ Marina Pretolani,¶ Véronique Flamand,* Michel Y. Braun,* Michel Goldman,* and Daniel Abramowicz†2

CD4 T cells play a crucial role in the acute rejection of MHC class II-disparate skin allografts, mainly by Fas/Fas ligand-mediated cytotoxicity. Because recent observations indicate that eosinophils may be found within allografts rejected by CD4 T cells, we evaluated the role played by IL-5, the main eosinophil growth factor, and by eosinophils in the rejection of MHC class II-disparate skin grafts. C57BL/6 mice rapidly rejected MHC class II-disparate bm12 skin grafts. Rejected skins contained a dense, aggressive eosinophil infiltrate. Lymphocytes isolated from lymph nodes draining rejected bm12 skin were primed for IL-5 secretion, and IL-5 mRNA was present within rejected grafts. The IL-5/eosinophil pathway played an effector role in allograft destruction, because the rejection of bm12 skin was significantly delayed in IL-5-deficient mice as compared with wild-type animals. The role of the IL-5/eosinophil pathway was further investigated in MHC class II-disparate donor-recipient strains unable to establish Fas/Fas ligand interactions. Fas ligand-deficient *gld/gld* mice rejected bm12 skins, and bm12 mice rejected Fas-deficient *lpr/lpr* C57BL/6 skins. Neutralization of IL-5 prevented acute rejection in both combinations. We conclude that MHC class II-disparate skin allografts trigger an IL-5-dependent infiltration of eosinophils that is sufficient to result in acute graft destruction. *The Journal of Immunology,* 1999, 163: 3778–3784.

A major role is played by CD4-positive T cells in the distinct effector pathways that lead to organ allograft rejection. First, CD4 T cells themselves may display direct cytotoxicity toward cells expressing MHC class II alloantigens (1). This pathway of cytotoxicity involves interactions between Fas ligand (FasL)[3] on CD4 lymphocytes and its counterreceptor Fas on allogeneic targets (2, 3). Second, CD4 T cells are the main producers of IL-2 and other cytokines critical for the clonal expansion of alloreactive CD8 cytotoxic cells (4). Third, they provide help to B cells to produce alloantibodies (5, 6). Fourth, CD4 T cells are able to recruit and activate macrophages within the allograft, leading to a delayed type hypersensitivity (DTH) alloreaction (7–9). Activated macrophages release toxic molecules, including oxygen radicals, TNF-α, and enzymes that contribute to graft damage (10–15). Recent observations suggest that there might be yet another effector mechanism of CD4-dependent allograft destruction involving eosinophils (16–18). Indeed, acutely rejected cardiac allografts from CD8-depleted recipient mice were infiltrated by numerous eosinophils (17). Along the same line, eosinophils were prominent within murine skin allografts rejected by alloreactive CD4 lines secreting IL-5, the major eosinophil growth factor (19–21). Activated eosinophils produce several toxic molecules such as major basic protein and eosinophil cationic protein (22) that, like macrophage products, may damage the allograft. However, whether eosinophils are just innocent bystanders or whether they play an effector role in allograft rejection has not been elucidated yet.

Here, we first show that MHC class II-disparate bm12 skin allografts rejected by C57BL/6 mice display a massive eosinophil infiltrate. The causal role of IL-5 was investigated by performing grafts on IL-5-deficient mice and by injection of neutralizing anti-IL-5 Abs in donor-recipient strains unable to establish Fas/FasL interactions.

Materials and Methods

Mice

C57BL/6 and BALB/c mice were obtained from IFFA CREDO (Brussels, Belgium). C57BL/6-*gld/gld* FasL-deficient mice, C57BL/6-*lpr/lpr* Fas-deficient mice, and C57BL/6.CH-2^{bm12} (bm12) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). C57BL/6 IL-5-deficient mice (20) were kindly provided by Dr. M. Kopf, Basel Institute for Immunology, Basel, Switzerland.

Skin grafting

Skin grafts ~1 cm in diameter were prepared from tails of female mice and grafted onto the flanks of the recipients according to an adaptation of the method of Billingham and Medawar (23). Petroleum gauze was placed over the graft, and sticking plaster was applied around the trunk. The bandages were removed after 10 days, and the grafts were monitored daily until day 30. Skins were considered rejected when complete epithelial breakdown had occurred. C57BL/6-*gld/gld* FasL-deficient mice were always grafted before 6 wk of age.

Ab preparation and in vivo treatments

Anti-CD4 (clone GK1.5), anti-CD8 (clone H35), anti-IL-5 (clone TRFK-5), and isotype control mAbs were produced as ascites in nude mice as previously described (24, 25). The mAb concentrations of ascites were determined by ELISA using anti-rat IgG1 mAb from LO-IMEX, University of Louvain, Louvain, Belgium. CD4- and CD8-positive cells were depleted with the IgG2b rat anti-murine CD4 mAb GK1.5 or the IgG2b rat anti-murine CD8 mAb H35, respectively. Animals received i.p. injections of 1 mg of either mAb 4 days before grafting; on the day of grafting, and then every 10 days until the end of the experiment. Flow cytometry analysis (FACScalibur, Becton Dickinson, Mountain View, CA) performed on the day of sacrifice with the use of PE-conjugated anti-CD4 (PharMingen, San Diego, CA, clone RM4-4) or anti-CD8 mAb (PharMingen, clone 53-6.7) showed <1% of corresponding T cell populations in lymph nodes. IL-5 was blocked in vivo by repeated i.p. injections of 1 mg of the IgG1 rat anti-mouse IL5 mAb, TRFK-5 (25) according to the following schedule: 1 day before grafting; 6 days after transplantation; then every 10 days until day 30. Control mice received the isotype-matched anti-DNP rat IgG1 mAb (LO-DNP-2, kindly provided by Dr. H. Bazin, Experimental Immunology Unit, Université Catholique de Louvain, Louvain, Belgium), according to the same schedule.

Histological studies

Skin graft histology was performed on tissue sections stained with hematoxylin and eosin, after paraffin embedding. The number of eosinophils infiltrating the graft was quantified by a pathologist unaware of the treatment groups. This was done by averaging the number of eosinophils present in at least three distinct high power fields (0.0025 mm^2 across the graft).

Production of cytokines in MLC

Cells from lymph nodes draining the skin allografts were used as responder cells (5×10^6/well) and seeded with 5×10^6 irradiated (2000 rad) bm12 spleen cells (stimulators) in 48-well flat-bottom plates (150687, Nunc, Roskilde, Denmark). Culture medium consisted of RPMI 1640 supplemented with 20 mM HEPES, 2 mM glutamine, 1 mM nonessential amino acids, 5% heat-inactivated FCS, sodium pyruvate, and 2-ME. Supernatants were harvested after 48–72 h of culture for determination of IFN-γ levels using ELISA DuoSet (Genzyme, Cambridge, MA). IL-5 was quantified by an enzyme immunometric assay, as previously described (26). The lower limits of detection of these assays were 30 pg/ml for IFN-γ and 5 pg/ml for IL-5.

CTL

Responder cells obtained from paraaortic and mesenteric lymph nodes were depleted in vitro of CD8 cells by incubation with the rat anti-mouse CD8 mAb (H35) followed by addition of rabbit complement. Depletion was confirmed by flow cytometry analysis (FACScalibur, Becton Dickinson, Mountain View, CA) with the use of PE-conjugated anti-CD8 mAb (PharMingen, clone 53-6.7). Of the remaining cells 5×10^6 were cultured with 5×10^6 irradiated (2000 rad) stimulator spleen cells in 24-well flat-bottom plates. Cultures were incubated at 37°C in 5% CO_2 in humidified air for 5 days. Target cells were prepared by incubation of 1×10^6 bm12 spleen cells with 30 μg/ml LPS (serotype 0111:B4, Sigma, Bornem, Belgium) in 2 ml medium for 2 to 3 days and pulsed overnight with [^{3}H]thymidine (Isobio, Fleurus, Belgium). Effector cells were harvested, washed, and plated at various E:T ratios in 96-well round-bottom plates containing 5×10^3 radiolabeled target cells. Each E:T ratio was performed in triplicate. After 3 h of incubation at 37°C, cultures were harvested on Unifilter plate, and residual radioactivity was measured with a Top Count microplate scintillation counter (Packard, Meriden, CT). The percentage of specific lysis was calculated according to the formula % specific lysis = [(spontaneous (cpm) − experimental (cpm))/spontaneous (cpm)] $\times$ 100, where "experimental" is labeled DNA retained in the presence of effector cells and "spontaneous" is labeled DNA retained in the absence of effector cells.

Cytokine mRNA analysis by reverse transcription PCR

Skin grafts from mice bearing either a syngeneic C57BL/6 transplant ($n = 4$) or an allogeneic bm12 graft undergoing acute rejection 15 days after transplantation ($n = 4$) were analyzed for cytokine mRNA. Syngeneic and allogeneic skin grafts were pooled, and total RNA was extracted using the guanidium thiocyanate method (Tripure, Boehringer Mannheim, Mannheim, Germany). Preparations of cDNA and PCR for IFN-γ, IL-5, and β-actin as housekeeping gene were performed by standard procedures (24). Briefly, PCR were performed in a total volume of 25 μl as follows: 1) denaturation: 4 min at 94°C; 2) amplification: 38 cycles for IL-5 and 35 cycles for IFN-γ and β-actin, respectively. Cycles were: 20 s at 94°C, 20 s
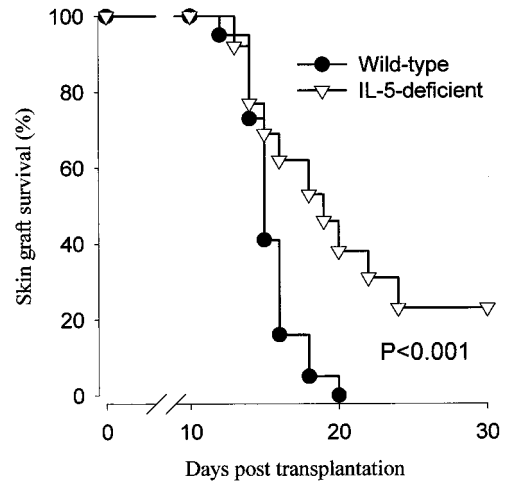

FIGURE 1. Delayed rejection of bm12 skin grafts on C57BL/6 IL-5-deficient mice. MHC class II-disparate bm12 skin tails were grafted on wild-type C57BL/6 (●) ($n = 37$) or IL-5-deficient C57BL/6 mice ($\triangledown$) ($n = 13$).

at 55°C, and 30 s at 72°C; and 3) extension: performed for 10 min at 72°C. Twelve microliters of each sample were run on a 2% agarose gel, stained with ethidium bromide, and visualized under UV light. PCR primers used consisted of the following: IFN-γ: sense primer 5′-GCTCTGAGACAAT GAACGCT-3′ and antisense 5′-AAAGAGATAATCTGGCTCTGC-3′; IL-5: sense primer 5′-TCACCGAGCTCTGTTGACAA-3′ and antisense 5′-CCACACTTCTCTTTTTGGCG-3′; and β-actin: sense primer 5′-TG GAATCCTGTGGCATCCATGAAAC-3′ and antisense 5′-TAAAACG CAGCTCAGTA-ACAGTCCG-3′.

Statistical analysis

Results are shown as a median with the range of values in parentheses. Graft survival curves and cytokine levels were compared by the log-rank test and by the Mann-Whitney nonparametric test, respectively. All comparisons were made two-tailed. In case mice did not reject their graft, they were given an arbitrary survival time of 30 days.

Results

MHC class II-disparate bm12 skin grafts rejected by wild-type C57BL/6 mice display a massive eosinophil infiltrate

Wild-type C57BL/6 mice rapidly reject MHC class II-disparate bm12 skin grafts (Fig. 1). Graft rejection occurred between day 12 and day 20 (median survival time, 15.0 days). Histological examination of rejected skin allografts revealed necrosis and sloughing of the epidermal layer (Fig. 2B). Although only rare eosinophils were present within syngeneic grafts, numerous eosinophils infiltrated the allogeneic skins (Table I). Eosinophils were particularly abundant along the epidermis and hair follicles, both structures that they heavily infiltrated (Fig. 2C). Many eosinophils showed degranulation, as indicated by the presence of their red granules within the interstitial tissue (Fig. 2D). This suggested that eosinophils contributed to tissue damage and rejection of the allogeneic skin.

Cytokine production by lymph nodes and intragraft detection of cytokine mRNAs

Because IL-5 is the main cytokine involved in the proliferation and differentiation of eosinophils, we searched for IL-5 production by lymphocytes from lymph nodes draining rejected bm12 skins, and we examined the intragraft expression of IL-5 mRNA. The presence of IFN-γ was also investigated, as this cytokine is known to be required for the rejection of bm12 transplants by C57BL/6 mice (27, 28). Lymph node cells from naive C57BL/6 mice produced increased amounts of both IL-5 and IFN-γ after stimulation with

FIGURE 2. Skin graft histology. *A,* Control, syngeneic C57BL/6 skin graft 30 days after transplantation. The dermis, below the epidermal layer (e), contains only rare inflammatory cells and some fibroblasts. Sebaceous glands (s) and hair follicles (h) display a normal aspect (original magnification, ×200). *B–D,* bm12 allogeneic skin graft rejected by C57BL/6 wild-type mice. In *B,* the epidermal layer (e) is undergoing sloughing. The dermis contains numerous inflammatory cells. The remnant of one hair follicle (h) can be seen engulfed in a dense inflammatory infiltrate (original magnification, ×200). *C,* The hair follicle is infiltrated by numerous eosinophils identified by their red cytoplasmic granules (original magnification, ×1000). *D,* degranulating eosinophils in a bm12 skin undergoing rejection. *E* and *F,* bm12 skin graft rejected by C57BL/6 FasL-deficient mice. There is sloughing of the epidermal layer (e), and the dermis contains an inflammatory infiltrate rich in eosinophils. Hair follicles (h) are undergoing destruction by eosinophils (*E* and *F*) (original magnification, ×200 and ×1000, respectively). *G,* Histology of bm12 skin grafted in C57BL/6 FasL-deficient mice injected with anti-IL-5 mAb. Skin graft was harvested 30 days after transplantation. The dermis contains only rare inflammatory cells and no eosinophils. As observed in control syngeneic grafts, hair follicles (h) and sebaceous glands (s) are preserved (original magnification, ×200).
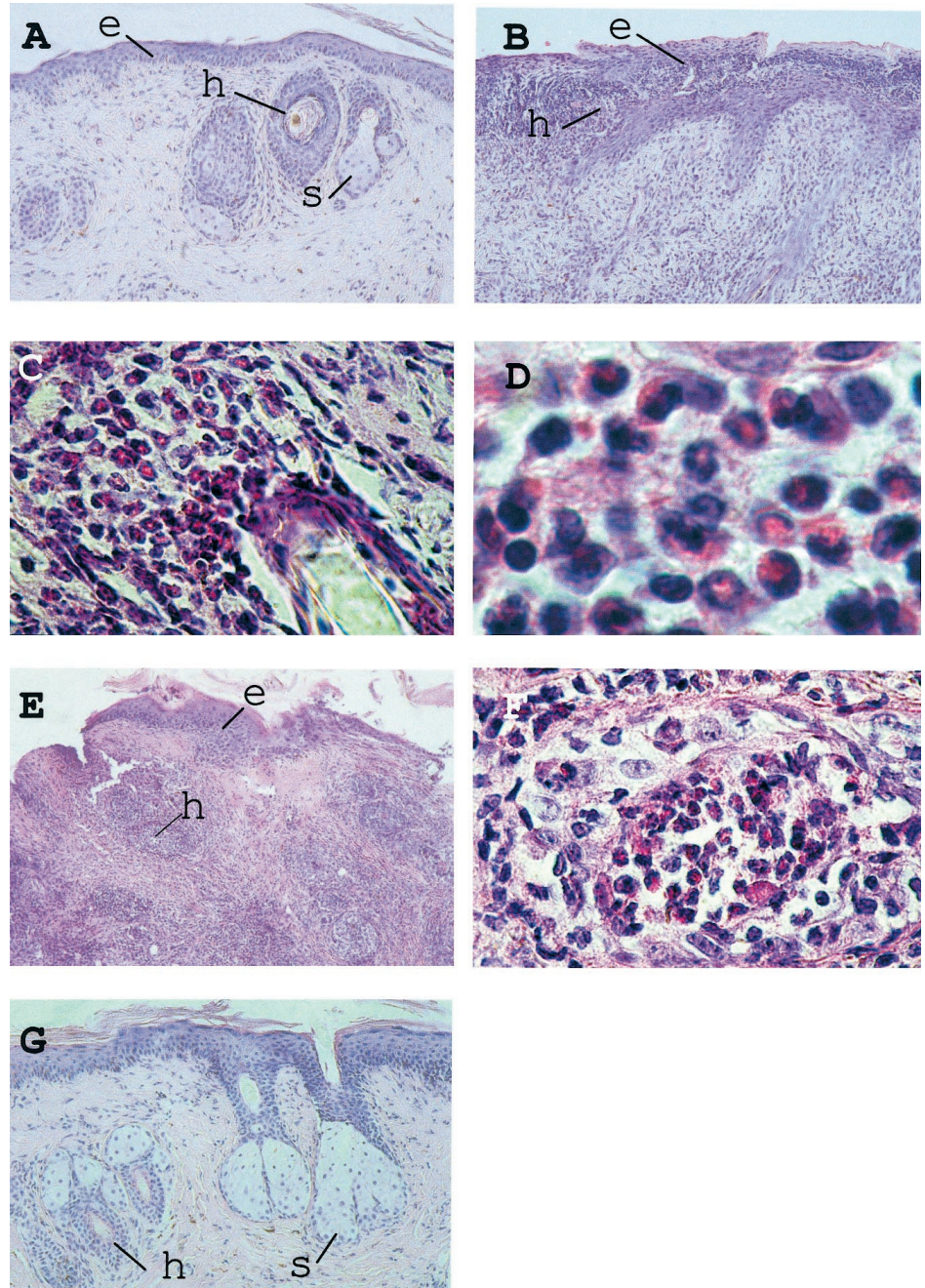
bm12 alloantigens in MLR ($p < 0.001$ vs syngeneic cultures) (Table II). As compared with these naive animals, lymphocytes from mice that have rejected a bm12 skin secreted about 5 times more IL-5 after stimulation with donor alloantigens. A modest priming for IFN-γ secretion was also seen (2-fold). This pattern of cytokine production was specific for the priming bm12 alloantigens, because no increased cytokine secretion was observed after stimulation with third-party BALB/c alloantigens (Table II). The

Table I. *Eosinophil infiltration of skin grafts*

	Donor-Type Skin Graft					
	Syngeneic		bm12-allogeneic			
Type of C57BL/6 recipient mAb treatment	Wild-type No	Wild-type No	IL-5-deficient No	FasL-deficient No	FasL-deficient Control mAb	FasL-deficient Anti-IL-5 mAb
No. of mice	5	8	7	11	11	10
No. of eosinophils/0.025 mm^2	1 (0–4)	68 (17–140)^a*	1 (0–30)†	70 (33–135)*	58 (23–110)*	1 (0–12)‡

^{a}*, $p < 0.0001$ compared with syngeneic skin grafts; †, $p < 0.0001$ compared with bm12 skins transplanted on wild-type animals; ‡$p < 0.0001$ compared with skins grafted onto FasL-deficient mice treated with the control mAb.

Table II. *Cytokine production in MLR[a]*

Responder C57BL/6 Mice	Priming with bm12 Skin Graft	Stimulator Cells	IL-5	IFN-γ
Wild-type	No	C57BL/6	115 (53–304)	55 (50–219)
	No	bm12	674 (359–3,200)	1,152 (402–2,716)
	No	BALB/c	370 (279–677)	2,070 (1,374–2,960)
	Yes	C57BL/6	233 (83–465)	50 (50–125)
	Yes	bm12	3,469 (1,835–8,628)†	2,178 (1,444–2,434)*
	Yes	BALB/c	500 (69–1,957)	1,780 (295–3,588)
FasL-deficient mice	No	C57BL/6	158 (95–414)	50 (50–170)
	No	bm12	492 (253–1,245)	849 (265–1,018)
	No	BALB/c	449 (315–831)	1,686 (150–1,986)
	Yes	C57BL/6	192 (69–414)	61 (50–283)
	Yes	bm12	4,768 (1,090–10,000)†	718 (126–1,140)
	Yes	BALB/c	506 (271–1,285)	1,148 (176–2,807)

[a] IL-5 and IFN-γ production (pg/ml) in MLR with syngeneic C57BL/6, donor-type allogeneic bm12, or third-party BALB/c stimulator spleen cells. Five mice were tested in each experimental condition. Results are representative of at least three separate experiments. *, $p < 0.05$ and †, $p < 0.001$ compared with unprimed mice of the same responder type.

presence of IL-5 and IFN-γ mRNA was analyzed for in allogeneic bm12 skin transplants at the time of rejection. Increased amounts of both cytokines mRNA were present within acutely rejected grafts as compared with syngeneic transplants (Fig. 3).

An IL-5/eosinophil pathway contributes to acute rejection of MHC class II-disparate grafts in wild-type C57BL/6 mice

To study the possible contribution of IL-5 to the rejection of MHC class II-disparate bm12 skins, grafts were performed on IL-5-deficient mice (Fig. 1). The rejection of bm12 grafts by IL-5-deficient mice was significantly delayed as compared with wild-type animals ($p < 0.001$, Fig. 1). Moreover, 3 of 13 IL-5-deficient mice were unable to reject the bm12 grafts, a finding not observed in wild-type animals ($n = 37$), which always experienced rapid rejection ($p = 0.018$). Histology of bm12 skin grafts rejected by IL-5-deficient mice revealed only very rare eosinophils (Table I).

IL-5 neutralization prevents acute rejection of MHC class II-disparate grafts in the absence of Fas/FasL interactions

The experiments described above indicate that an IL-5/eosinophil pathway contributes to the rejection of MHC class II-incompatible

skin grafts. The rejection that still occurs in the majority of IL-5-deficient mice is likely to be mediated by CD4 cytotoxic cells. To investigate the possible role played by the IL-5/eosinophil pathway in the rejection of bm12 allografts when CD4 cytotoxicity is absent, we first performed bm12 skin grafts in C57BL/6 FasL-deficient mice. As shown in Fig. 4, the large majority of FasL-deficient mice acutely rejected bm12 grafts, with a kinetics comparable with that of wild-type mice. In vitro experiments confirmed that CD4 T cells from FasL-deficient mice were unable to mount an anti-bm12 cytotoxic activity (Fig. 5). CD4 T cells from FasL-deficient mice were, however, still required for the rejection process as shown by T cell depletion experiments (Fig. 4). The rejection of bm12 skins by C57BL/6 FasL-deficient mice was associated with the presence of IL-5 and eosinophils similar to those observed in wild-type C57BL/6 mice. Indeed, bm12 skin grafts rejected by FasL-deficient mice displayed a massive eosinophil infiltration (Fig. 2F and Table I); and T cells from rejecting mice were primed for IL-5 production in MLR (Table II). The two bm12 skin allografts that
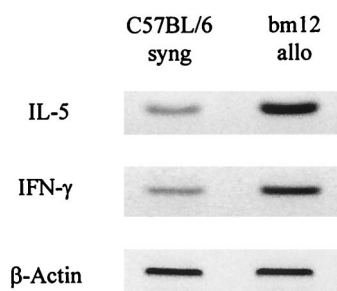

FIGURE 3. Cytokine gene expression within rejected allografts. IL-5 and IFN-γ mRNAs expression was compared within pooled syngeneic C57BL/6 grafts ($n = 4$) or bm12 allografts ($n = 4$) harvested at the time of rejection. The mRNA of the housekeeping gene β-actin is also shown. Similar results were obtained in two other separate experiments performed on pooled skin grafts ($n = 3$ to 4 per pool).
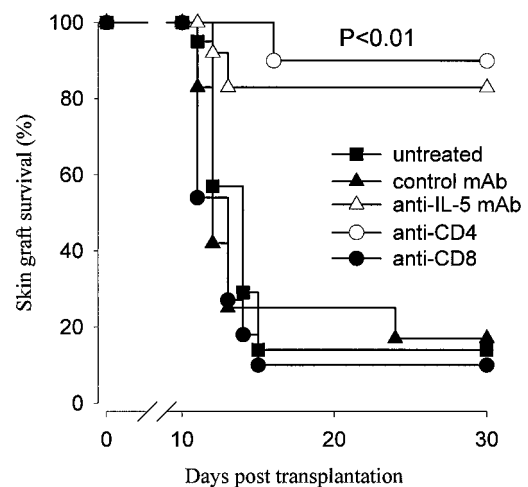

FIGURE 4. bm12 skin graft rejection by FasL-deficient mice. MHC class II-disparate bm12 skin tails were grafted on FasL-deficient mice left untreated (■; $n = 12$); injected with isotype-matched control rat IgG1 mAb (▲; $n = 12$); injected with rat IgG1 anti-IL-5 mAb (△; $n = 12$); injected with rat anti-CD8 mAb (●; $n = 11$); and injected with rat mAb anti-CD4 (○; $n = 10$).
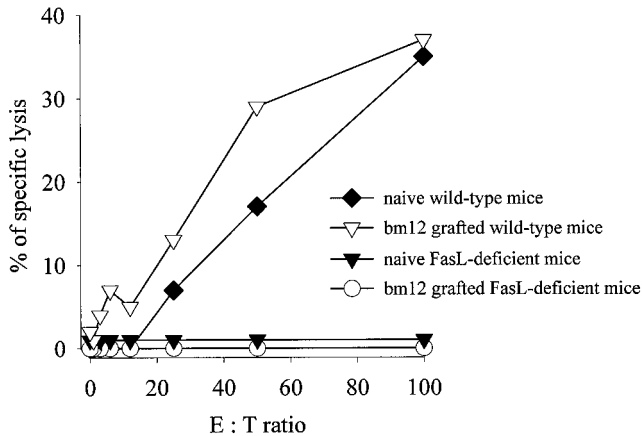
FIGURE 5. The generation of cytotoxic CD4 T cells is impaired in FasL-deficient mice. Lymph node cells from wild-type and FasL-deficient mice that were either left untreated (naive) or received a bm12 graft were depleted in vitro of CD8 cells and cultured with irradiated bm12 spleen cells. After 5 days of culture, anti-bm12 CTL activities were measured in a 3-h CTL assay. Each experimental group represents a pool of three mice. Similar results were obtained in three separate experiments.

were not rejected appeared normal, with no eosinophil infiltrate. The functional role played by IL-5 and eosinophils in the rejection of bm12 skin by FasL-deficient mice was tested by the administration of neutralizing anti-IL-5 Abs. As shown in Fig. 4, the majority of anti-IL-5-injected mice were unable to reject their transplants. At day 30 (day of the sacrifice), the tolerated grafts displayed an appearance comparable with that of syngeneic grafts and were devoid of eosinophils (Fig. 2 and Table I). FasL-deficient mice injected with the control mAb experienced rejection with a tempo equivalent to that of untreated FasL-deficient animals, and the rejected grafts were heavily infiltrated by eosinophils (Table I). To further confirm the ability of the IL-5/eosinophil pathway to trigger acute allograft rejection in the absence of Fas/FasL interactions, we performed MHC class II-disparate skin grafts from Fas-deficient C57BL/6-*lpr/lpr* mice on wild-type bm12 animals. bm12 mice, either untreated or after injections of the control rat IgG1 mAb, promptly rejected the Fas-deficient skins (Fig. 6),
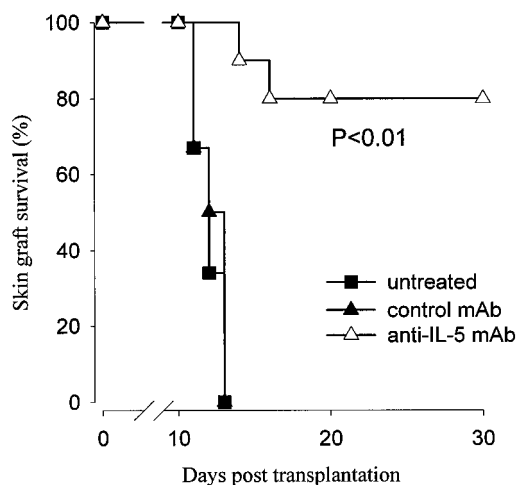

FIGURE 6. Fas-deficient (*lpr/lpr*) C57BL/6 skin graft rejection by bm12 mice. MHC class II-disparate Fas-deficient C57BL/6 skin tails were grafted on bm12 mice left untreated (■; $n = 10$); injected with isotype-matched control rat IgG1 mAb (▲; $n = 6$); and injected with rat IgG1 anti-IL-5 mAb (△; $n = 10$).

which histologically displayed a dense eosinophil infiltrate (not shown). Administration of the anti-IL-5 mAb prevented acute rejection in 80% of mice ($p < 0.01$) (Fig. 6).

Discussion

The main finding from these experiments is that IL-5 and eosinophils represent an effector pathway of the rejection of MHC class II-disparate skin grafts. Eosinophils are a prominent feature of bm12 skins acutely rejected by B6 animals. They are mainly concentrated along the dermoepidermal junction and hair follicles. Eosinophil degranulation was evident, as indicated by the presence of their red granules within graft interstitial tissue. These granules contain several molecules involved in eosinophil toxicity, such as neurotoxin, eosinophil cationic protein, and major basic protein (29, 30). These and other molecules are responsible for the ability of eosinophils to induce cytolysis and acute tissue damage after exposure to allergens or parasites (29, 30). As a matter of fact, activated eosinophils have recently been shown to play a critical role in the rejection of solid tumors in mice (31, 32).

Although the presence of eosinophils within acutely rejected allografts has been observed in other experimental settings (16, 17), their causal role in acute allograft rejection has not been established yet. The infiltration of tissues by eosinophils is critically dependent on the availability of IL-5. Indeed, experiments with IL-5-deficient mice or using neutralizing anti-IL-5 mAbs revealed the essential role of this cytokine in the proliferation and differentiation of eosinophils (19–21, 33, 34), as well as in their recruitment and activation within tissues (35). Therefore, our observations that lymph node cells draining rejected bm12 skins produced large amounts of IL-5 together with the presence of abundant amounts of IL-5 mRNA within rejected grafts readily explain the infiltration of the allograft by eosinophils. To address the possible causal role played by IL-5 in acute rejection, we grafted MHC class II-disparate bm12 skins onto IL-5-deficient C57BL/6 mice. Importantly for these experiments, the classical effector mechanisms that may also contribute to graft rejection such as Ab response and the generation of cytolytic T cells are normal in IL-5-deficient mice (20). Of course, one cannot exclude that blocking IL-5 might also inhibit the alloreactive response by other, as yet undefined mechanisms in addition to preventing eosinophil infiltration. Several IL-5-deficient mice did not experience rejection and maintained the donor bm12 graft in perfect condition for at least 30 days. The rejection that occurred in the other IL-5-deficient animals was significantly delayed as compared with wild-type mice. Taken together, these data indicate that eosinophils represent one effector pathway that contributes to the rejection of MHC class II-disparate skin. It is important to stress that the present observations were made in the bm12→BL/6 strain combination, which differs for only 3 amino acids within the Ia MHC class II Ags. Although IL-5 and eosinophils have also been found within rejected allografts in strains differing at other MHC class II Ags (16, 17), the functional role played by the IL-5/eosinophil pathway in these combinations remains to be defined.

The rejection that still occurs in many IL-5-deficient mice may probably involve cytotoxic anti-MHC class II alloreactive CD4^{+} T cells. Indeed, transfer experiments have shown that such cells are able to reject a bm12 skin transplanted on nude mice (1). The cytotoxic activity developed by CD4 T cells results from the interactions between FasL and its counterreceptor Fas on allogeneic targets (2, 3). In the skin, keratinocytes are known to express Fas in the basal state and may therefore become sensitive to FasL-mediated apoptosis induced by alloreactive CD4 cytotoxic T cells (36–38). To address the ability of the IL-5/eosinophil pathway to

induce rejection of MHC class II-incompatible grafts when CD4 cytotoxicity is deficient, we grafted MHC class II-disparate skins in two strain combinations unable to establish productive Fas/FasL interactions. In this context, the eosinophilic rejection could be prevented by IL-5 neutralization. As in wild-type mice, T cells from FasL-deficient animals specific for bm12 MHC class II alloantigen were indeed primed for IL-5 production. Because CD8 T cells were shown in different settings to down-regulate tissue eosinophilia and IL-5 synthesis by CD4 T cells (17, 39–41), the priming for IL-5 production in the present model might be related to the lack of disparity for MHC class I Ags. Indeed, in vivo cell depletion experiments confirmed that CD8 T cells were not involved in the rejection process. Interestingly, the anti-bm12 response was not of the Th2 type in that lymphocytes from mice grafted with bm12 skin also produced significant amounts of IFN-γ in MLR. A priming for IFN-γ was observed after bm12 skin grafting in wild-type recipients but not in FasL-deficient mice. The reason for this difference is not known, but it might be because IL-4, a cytokine that may inhibit IFN-γ production (42), was detected in increased amounts with lymphocytes from FasL-deficient mice as compared with wild-type mice after MLR with bm12 alloantigens (A. Le Moine, unpublished results). As recently demonstrated in a model of tumor rejection, IFN-γ and IL-5 may synergize in the induction of tissue damage involving eosinophils (31). If the role of IFN-γ in the rejection of MHC class II-disparate skin allografts has been well established (27, 28), our observations provide the first evidence that IL-5 is a key mediator of rejection in the absence of antidonor cytotoxicity. Because eosinophil infiltrates are often found in biopsies of liver or kidney allografts during rejection episodes (43–46), we suggest that a similar IL-5/eosinophil pathway might contribute to certain forms of rejection in clinical transplantation.

Acknowledgments

References

1. Rosenberg, A. S., and A. Singer. 1992. Cellular basis of skin allograft rejection: an in vivo model of immune-mediated tissue destruction. *Annu. Rev. Immunol.* 10:333.

2. Van Parijs, L., and A. K. Abbas. 1996. Role of Fas-mediated cell death in the regulation of immune responses. *Curr. Opin. Immunol.* 8:355.

3. Hahn, S., R. Gehri, and P. Erb. 1995. Mechanism and biological significance of CD4-mediated cytotoxicity. *Immunol. Rev.* 146:57.

4. Kalams, S. A., and B. D. Walker. 1999. The critical need for CD4 help in maintaining effective cytotoxic T lymphocyte responses. *J. Exp. Med.* 188:2199.

5. Gonzalez, M., R. Merino, A. L. Gonzalez, and J. Merino. 1995. The ability of B cells to participate in allogeneic cognate T-B cell interactions in vitro depends on the presence of CD4+ T cells during their development. *J. Immunol.* 155:1091.

6. DeKruyff, R. H., L. V. Rizzo, and D. T. Umetsu. 1993. Induction of immunoglobulin synthesis by CD4+ T cell clones. *Semin. Immunol.* 5:421.

7. Valujskikh, A., D. Matesic, A. Gilliam, D. Anthony, T. M. Haqqi, and P. S. Heeger. 1998. T cells reactive to a single immunodominant self-restricted allopeptide induce skin graft rejection in mice. *J. Clin. Invest.* 101:1398.

8. Goto, M., Y. Yamaguchi, O. Ichiguchi, N. Miyanari, E. Akizuki, F. Matsumura, T. Matsuda, K. Mori, and M. Ogawa. 1997. Phenotype and localization of macrophages expressing inducible nitric oxide synthase in rat hepatic allograft rejection. *Transplantation* 64:303.

9. Sirak, J., C. G. Orosz, E. Wakely, and A. M. VanBuskirk. 1997. Alloreactive delayed-type hypersensitivity in graft recipients: complexity of responses and divergence from acute rejection. *Transplantation* 63:1300.

10. Dalloul, A., K. Chmouzis, K. Ngo, and W. Fung-Leung. 1996. Adoptively transferred CD4+ lymphocytes from CD8−/− mice are sufficient to mediate the rejection of MHC class II or class I disparate skin grafts. *J. Immunol.* 156:4114.

11. Sekine, Y., L. K. Bowen, K. M. Heidler, N. Van Rooijen, J. W. Brown, O. W. Cummings, and D. S. Wilkes. 1997. Role of passenger leukocytes in allograft rejection: effect of depletion of donor alveolar macrophages on the local production of TNF-α, T helper 1/T helper 2 cytokines, IgG subclasses, and pathology in a rat model of lung transplantation. *J. Immunol.* 159:4084.

12. Gomez Flores, R., C. Rodriguez Padilla, R. T. Mehta, L. Galan Wong, E. Mendoza Gamboa, and R. Tamez Guerra. 1997. Nitric oxide and TNF-α production by murine peritoneal macrophages activated with a novel 20-kDa protein isolated from *Bacillus thuringiensis* var. *thuringiensis* parasporal bodies. *J. Immunol.* 158:3796.

13. Nadeau, K. C., H. Azuma, and N. L. Tilney. 1995. Sequential cytokine dynamics in chronic rejection of rat renal allografts: roles for cytokines RANTES and MCP-1. *Proc. Natl. Acad. Sci. USA* 92:8729.

14. Yang, X., N. Chowdhury, B. Cai, J. Brett, C. Marboe, R. R. Sciacca, R. E. Michler, and P. J. Cannon. 1994. Induction of myocardial nitric oxide synthase by cardiac allograft rejection. *J. Clin. Invest.* 94:714.

15. Schook, L. B., H. Albrecht, P. Gallay, and C. V. Jongeneel. 1994. Cytokine regulation of TNF-α mRNA and protein production by unprimed macrophages from C57BL/6 and NZW mice. *J. Leukocyte Biol.* 56:514.

16. Matesic, D., A. Valujskikh, E. Pearlman, A. W. Higgins, A. C. Gilliam, and P. S. Heeger. 1998. Type 2 immune deviation has differential effects on alloreactive CD4+ and CD8+ T cells. *J. Immunol.* 161:5236.

17. Chan, S. Y., L. A. DeBruyne, R. E. Goodman, E. J. Eichwald, and D. K. Bishop. 1995. In vivo depletion of CD8+ T cells results in Th2 cytokine production and alternate mechanisms of allograft rejection. *Transplantation* 59:1155.

18. Martinez, O. M., N. L. Ascher, L. Ferrell, J. Villanueva, J. Lake, J. P. Roberts, and S. M. Krams. 1993. Evidence for a nonclassical pathway of graft rejection involving interleukin 5 and eosinophils. *Transplantation* 55:909.

19. Nakajima, H., I. Iwamoto, S. Tomoe, R. Matsumura, H. Tomioka, K. Takatsu, and S. Yoshida. 1992. CD4+ T-lymphocytes and interleukin-5 mediate antigen-induced eosinophil infiltration into the mouse trachea. *Am. Rev. Respir. Dis.* 146:374.

20. Kopf, M., F. Brombacher, P. D. Hodgkin, A. J. Ramsay, E. A. Milbourne, W. J. Dai, K. S. Ovington, C. A. Behm, G. Kohler, I. G. Young, and K. I. Matthaei. 1996. IL-5-deficient mice have a developmental defect in CD5+ B-1 cells and lack eosinophilia but have normal antibody and cytotoxic T cell responses. *Immunity* 4:15.

21. Foster, P. S., S. P. Hogan, A. J. Ramsay, K. I. Matthaei, and I. G. Young. 1996. Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *J. Exp. Med.* 183:195.

22. Kroegel, C., J. C. Virchow, Jr., W. Luttmann, C. Walker, and J. A. Warner. 1994. Pulmonary immune cells in health and disease: the eosinophil leucocyte (Part I). *Eur. Respir. J.* 7:519.

23. Billingham, R., and Medawar PB. 1951. The technique of free skin grafting in mammals. *J. Exp. Biol.* 28:385.

24. Donckier, V., M. Wissing, C. Bruyns, D. Abramowicz, M. Lybin, M. L. Vanderhaeghen, and M. Goldman. 1995. Critical role of interleukin 4 in the induction of neonatal transplantation tolerance. *Transplantation* 59:1571.

25. Schumacher, J. H., A. O'Garra, B. Shrader, A. van Kimmenade, M. W. Bond, T. R. Mosmann, and R. L. Coffman. 1988. The characterization of four monoclonal antibodies specific for mouse IL-5 and development of mouse and human IL-5 enzyme-linked immunosorbent. *J. Immunol.* 141:1576.

26. Zuany Amorim, C., C. Creminon, M. C. Nevers, M. A. Nahori, B. B. Vargaftig, and M. Pretolani. 1996. Modulation by IL-10 of antigen-induced IL-5 generation, and CD4+ T lymphocyte and eosinophil infiltration into the mouse peritoneal cavity. *J. Immunol.* 157:377.

27. Rosenberg, A. S., D. S. Finbloom, T. G. Maniero, P. H. Van der Meide, and A. Singer. 1990. Specific prolongation of MHC class II disparate skin allografts by in vivo administration of anti-IFN-γ monoclonal antibody. *J. Immunol.* 144:4648.

28. Russell, P. S., C. M. Chase, H. J. Winn, and R. B. Colvin. 1994. Coronary atherosclerosis in transplanted mouse hearts. III. Effects of recipient treatment with a monoclonal antibody to interferon-γ. *Transplantation* 57:1367.

29. Gleich, G. J., C. R. Adolpson, and K. M. Leiferman. 1993. The biology of the eosinophilic leukocyte. *Annu. Rev. Med.* 44:85.

30. Rothenberg, M. E. 1998. Eosinophilia. *N. Engl. J. Med.* 338:1592.

31. Hung, K., R. Hayashi, A. Lafond-Walker, C. Lowenstein, D. Pardoll, and H. Levitsky. 1998. The central role for CD4+ T cells in the anti-tumor immune response. *J. Exp. Med.* 1988:2357.

32. Tepper, R. I., R. L. Coffman, and P. Leder. 1992. An eosinophil-dependent mechanism for the antitumor effect of interleukin-4. *Science* 257:548.

33. Sanderson, C. J. 1992. Interleukin-5, eosinophils, and disease. *Blood* 79:3101.

34. Coffman, R. L., B. W. Seymour, S. Hudak, J. Jackson, and D. Rennick. 1989. Antibody to interleukin-5 inhibits helminth-induced eosinophilia in mice. *Science* 245:308.

35. Mould, A. W., K. I. Matthaei, I. G. Young, and P. S. Foster. 1997. Relationship between interleukin-5 and eotaxin in regulating blood and tissue eosinophilia in mice. *J. Clin. Invest.* 99:1064.

36. Backer, M. B., N. H. Altman, E. R. Podack, and R. B. Levy. 1996. The role of cell-mediated cytotoxicity in acute GVHD after MHC-matched allogeneic bone marrow transplantation in mice. *J. Exp. Med. 183:2646.*

37. Linder, G., V. A. Botchkarev, N. V. Botchkareva, G. Ling, C. van der Veen, and R. Paus. 1997. Analysis of apoptosis during hair follicle regression. *Am. J. Pathol. 151:1601.*

38. Viard, I., P. Wehrli, R. Bullani, P. Schneider, N. Holler, D. Salomon, T. Hunziker, J. H. Saurat, J. Tschopp, and L. E. French. 1998. Inhibition of toxic epidermal necrolysis by blockade of CD95 with human intravenous immuno-globulin. *Science 282:490.*

39. Hussell, T., C. J. Baldwin, A. O'Garra, and P. J. Openshaw. 1997. CD8⁺ T cells control Th2-driven pathology during pulmonary respiratory syncytial virus in-fection. *Eur. J. Immunol. 27:3341.*

40. Hussell, T., A. Georgiou, T. E. Sparer, S. Matthews, P. Pala, and P. J. M. Openshaw. 1998. Host genetic determinants of vaccine-induced eosino-philia during respiratory syncytial virus infection. *J. Immunol. 161:6215.*

41. Tang, H., C. sharp, K. P. Peterson, and H. Braley-Mullen. 1998. IFN-gamma-deficient mice develop severe granulomatous experimental autoimmune thyroi-ditis with eosinophil and infiltration in thyroids. *J. Immunol. 160:5105.*

42. Constant, S. L., and K. Bottomly. 1997. Induction of Th1 and Th2 CD4⁺ T cell responses: the alternative approaches. *Annu. Rev. Immunol. 15:297.*

43. Martinez, O. M., J. C. Villanueva, J. Lake, J. P. Roberts, N. L. Ascher, and S. M. Krams. 1993. IL-2 and IL-5 gene expression in response to alloantigen in liver allograft recipients and in vitro. *Transplantation 55:1159.*

44. de Groen, P. C., G. M. Kephart, G. J. Gleich, and J. Ludwig. 1994. The eosinophil as an effector cell of the immune response during hepatic allograft rejection. *Hepatology 20:654.*

45. Nolan, C. R., K. P. Saenz, C. A. Thomas, and K. D. Murphy. 1995. Role of the eosinophil in chronic vascular rejection of renal allografts. *Am. J. Kidney Dis. 26:634.*

46. Ten, R. M., G. J. Gleich, K. E. Holley, J. D. Perkins, and V. E. Torres. 1989. Eosinophil granule major basic protein in acute renal allograft rejection. *Trans-plantation 47:959.*