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Homing of In Vitro-Generated Donor Antigen-Reactive CD4+ T Lymphocytes to Renal Allografts Is $\alpha_4\beta_1$ But Not $\alpha_L\beta_2$ Integrin Dependent

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The extravasation and sequestration of Ag-reactive T lymphocytes into vascularized organ allografts depend on a cascade of complex interactions among circulating lymphocytes, endothelial cells, and extracellular matrix proteins. Ag-activated donor-specific CD4 T cells are major initiators and effectors in the allograft rejection response. Interfering with the intragraft homing of activated CD4 T cells may represent a novel therapeutic approach in transplant recipients. We have developed a FACS-based short-term homing assay that allows tracing in vitro-generated Ag-reactive CD4 T cells after adoptive transfer in test rat recipients. Allospecific cell lines were preincubated with anti-$\alpha_4\beta_1$ or anti-$\alpha_L\beta_2$ mAb, because of enhanced expression of both integrin receptors after alloactivation. The pretreated LewisBN lymphocytes were carboxyfluorescein diacetate succinimidyl ester labeled and adoptively transferred into Lewis rat recipients of Brown Norway kidney allografts. The injection of equal numbers of Immunology, a novel strategy in preventing local intragraft recruitment of Ag-reactive CD4 T cells in transplant recipients. The Journal of treatment with anti-$\alpha_4\beta_1$ mAb diminished intragraft infiltration of adoptively transferred T cells by 85% in a donor-specific fashion. In contrast, treatment with anti-$\alpha_L\beta_2$ mAb did not affect intragraft cell sequestration. Hence, blocking $\alpha_4\beta_1$ integrin interactions represents a novel strategy in preventing local intragraft recruitment of Ag-reactive CD4 T cells in transplant recipients. The Journal of Immunology, 2001, 166: 596–601.

Donor-reactive CD4 T lymphocytes are major initiators and effectors in acute and chronic allograft rejection (1, 2). The immune rejection cascade starts immediately after organ transplantation, and involves activation of intragraft APC that migrate to the host-draining lymph nodes (LN) and/or spleen. The APC prime donor-reactive T cells for cytokine-dependent clonal expansion and differentiation into effector T cells. The homing pattern of effector cells is distinct from that of naive T cells (3). Different adhesion molecule expression profiles allow recirculating cells to migrate into tissues, particularly into the graft, and interacting with adhesion molecule counterparts or chemokines expressed on inflamed endothelial cells and extracellular matrix (ECM). The role of the Ag in the effector cell homing into the target tissue remains relatively ill-defined. In a rat model of experimental autoimmune encephalomyelitis, it was shown that both autoantigen (myelin basic protein)-specific and unrelated activated T cells transmigrate into the brain, but only myelin basic protein-specific T cells persisted there for more than 24 h (4), suggesting that Ag is involved in trapping of T cells in the tissue. The role of alloantigen specificity in homing to the graft is less well studied.

In the past, conventional immunosuppressive drugs were designed either to deplete circulating T cells (ATG, irradiation, OKT3) or to inhibit the process of Ag priming, cytokine production/action, and clonal expansion (calcineurin inhibitors, antiproliferative drugs, corticosteroids, rapamycin) of harmful alloreactive cells. However, interfering with the homing of alloreactive T cells and other effector cells into the graft is a new approach for preventing and combating graft-deteriorating immune processes. The inhibitory impact of conventional immunosuppressive drugs can be easily screened in vitro by measuring immune features such as diminished NF-κB activation, free Ca$^{2+}$ levels, cytokine production, or proliferation after T cell activation (5). In contrary, the effects of agents that modulate the homing are more difficult to address.

In the syngeneic situation, the highly inflamed transplant, caused by ischemia/reperfusion injury, attracts mainly polymorphonuclear cells and monocytes/macrophages; Ag-activated lymphocytes are only secondary in the pathogenesis of the very early ultimate graft loss. Indeed, short-term treatment with agents that bind to homing receptors (e.g., P-selectin glycoprotein ligand-1Ig) prevents ischemia/reperfusion injury in syngeneic grafts, stressing the importance of early Ag-nonspecific immune response (6). Initial Ag-nonspecific graft injury and alloantigen priming of T cells in the secondary immune organs promote accumulation of alloreactive effector cells in the graft, which in turn initiates acute rejection. CD4 T cell-derived cytokines (e.g., IFN-γ) and surface receptors (e.g., CD40 ligand) also attract and activate monocytes/macrophages, thereby initiating localized delayed-type hypersensitivity reaction (7, 8). Although continuous immunosuppression usually prevents early acute rejection, increased Ag-specific Th...
frequencies in peripheral blood reflect donor-directed reactivity during later rejection episodes in transplant recipients (9, 10).

The rolling, firm adhesion, and extravasation of activated lymphocytes is a highly controlled multistep process (11). In the case of activated type 1 T cells, P- and E-selectin ligands are believed to be responsible for the first loose rolling attachment (12). This slower movement allows the interaction of cellular chemokine and integrin receptors as well as CD44 with their endothelial counterparts. This firm adhesion promotes the diapedesis of lymphocytes into the tissue, where ECM proteins provide framework for sequestration and intercellular migration (13). Integrin-, selectin-, chemokine-, and CD44-dependent steps in T cell homing represent potential targets in blocking the sequestration of activated Ag-reactive T lymphocytes into the allografts. The \( \alpha \beta_1 \) (VLA-4) and \( \alpha \beta_2 \) (LFA-1) integrins are of particular importance. Indeed, both have specific endothelial counterreceptors (VCAM-1, ICAM-1/2/3, respectively), besides their affinity toward common binding sites of ECM proteins (e.g., RGD motif) and specific binding sites (e.g., CS-1 splicing motif on fibronectin) (14). Moreover, \( \alpha \beta_2 \) is a versatile integrin, which can support both rolling and firm adhesion of leukocytes to VCAM-1 (15); its binding ligand affinity is significantly up-regulated in activated (CD25high) CD4 lymphocytes (16). Interestingly, mAbs against \( \alpha \beta_2 \) integrin have been demonstrated to inhibit the onset of experimental autoimmune encephalomyelitis initiated by Thy1 lymphocytes specific for the myelin basic protein (17). mAb targeting adhesion molecules represent an interesting tool for studying cellular interactions in T cell homing in vivo. However, adhesion molecules are not only involved in homing of T cells, but also in their activation by delivering costimulatory signals (18) as well as in regulating Th1-type cytokine secretion (19). This makes the interpretation of their in vivo action difficult. The use of in vitro preactivated T cells for in vivo homing studies circumvents this problem. Moreover, the ability of resting cells to sequester at the graft site is limited as compared with that of alloactivated cells (20).

In the present study, we investigated the role of \( \alpha \beta_1 \) and \( \alpha \beta_2 \) integrin expression in the trafficking of donor-specific CD4 T cells in rat renal allograft recipients. In addition, the impact of the TCR specificity on CD4 lymphocyte recruitment to renal allografts was addressed.

**Materials and Methods**

**Animals and grafting techniques**

Inbred male adult rats (200–250 g) were used (Harlan Sprague Dawley, Indianapolis, IN). Lewis (LEW, RT1a) or Brown Norway (BN, RT1b) kidneys were transplanted into the abdominal great vessels of LEW recipients by using standard microvascular techniques. Wistar Furth (WF, RT1a) rats served as third-party donors to generate control cell lines.

**Generation of alloantigen-specific T lymphocytes**

The generation of Ag-reactive T lymphocytes has been described elsewhere (21). Briefly, LN cells from LEW rats (3 × 106 cells/well) were incubated with \( \gamma \)-irradiated (5000 rad) LN cells from BN donors (3 × 106 cells/well). The cells were grown in T cell medium (TCM) containing DMEM with 2 mM l-glutamine, 2 mM t-asparagine, antibiotics, and 2% heat-inactivated autologous LEW serum. After 4 days in culture, T cell blasts were harvested using rat Ficoll gradient centrifugation and propagated for 6–8 days in IL-2-conditioned medium. This medium was based on TCM supplemented with supernatant produced by Con A-stimulated rat splenocytes. Contaminating Con A was removed by addition of methyl mannosylpyranoside (Sigma, St. Louis, MO). For the following restimulation, resting T lymphocytes were incubated with \( \gamma \)-irradiated (5000 rad) syngeneic LEW thymocytes (2 × 105/well) in the presence of \( \gamma \)-irradiated (5000 rad) BN thymocytes (4 × 105 cells/well). T lymphocyte blasts were transferred into IL-2-conditioned medium and propagated. Alloantigen specificity of T lymphocytes was tested with a standard proliferation assay measuring [3H]TdR incorporation.

**FACS analysis**

Phenotype analysis of cell lines was performed by using mouse mAbs against rat CD4, CD8, CD45RC, and L-selectin (BioSource International, Camarillo, CA; PharMingen, San Diego, CA). mAbs against \( \alpha \beta_1 \) and \( \alpha \beta_2 \) integrin receptors were directly labeled with Alexa Fluor 488 Protein Labeling kit according to manufacturer’s protocol (Molecular Probes, Eugene, OR). For indirect Ab staining of \( \alpha \beta_1 \) and \( \alpha \beta_2 \)-stimulated lymphocytes, cells were incubated at 4°C for 20 min in 0.1 ml PBS with 1% goat serum and a PE-conjugated goat anti-mouse Ig (Zymed, San Francisco, CA). After washing twice in PBS, cells were analyzed by a FACScan (Becton Dickinson, Franklin Lakes, NJ), and data were evaluated with FCS-Express software (De Novo Software, Thornhill, Ontario, Canada).

In intracellular cytokine staining, the cells were harvested 3 days after alloantigen-dependent restimulation (IFN-\( \gamma \)) or after stimulation with PMA (10 ng/ml) and ionomycin (400 ng/ml) at 2.5 × 105/ml for 4 h, respectively (IL-4). GolgiStop (4 \( \mu \)l of ml; PharMingen) was added for the last 4 h. For intracellular cytokine staining, the cells were restimulated with PMA (10 ng/ml) and ionomycin (400 ng/ml) at 2.5 × 105/ml for 4 h in the presence of GolgiStop (4 \( \mu \)l/ml; PharMingen). The stimulated cells were collected and fixed/permeabilized using the CytoStain kit (PharMingen). Normal rat IgG (10 \( \mu \)g) was added to 1 million cells to block nonspecific binding sites, followed by mouse anti-rat IFN-\( \gamma \) (DB-1) or IL-4 (OX-81) mAb conjugated with R-PE (PharMingen). The stained cells were washed and then analyzed by a three-color FACS. CD4+ lymphocytes were gated for cytokine expression. PMA + ionomycin-driven stimulation does not alter intracellular cytokine expression profile seen after allostimulation.

**TCR V\( \beta \) RT-PCR**

RNA was extracted from lymphocytes using a Qiagen RNase Mini kit (Qiagen, Valencia, CA). A total of 0.2–1 \( \mu \)g of RNA was reverse transcribed into cDNA (40 \( \mu \)l) by 20 U of Moloney murine leukemia virus reverse transcriptase (Roche Molecular Biochemicals, Indianapolis, IN). A total of 0.5 \( \mu \)l of cDNA was amplified in 25 \( \mu \)l using a fixed downstream primer (Cb1) derived from TCR V\( \beta \)-chain C region and one of the 22 different/specific upstream primers. The PCR mixture was set up as follows: cDNA, 0.4 \( \mu \)M of each primers, 0.2 mM of each dNTP, 2 mM MgCl\(_2\), and 0.725 U of AmpliTaq Gold (Perkin-Elmer, Foster City, CA) in 1 \( \times \) GeneAmp buffer II. Amplifications were conducted in a GeneAmp PCR System 9700 (Perkin-Elmer). The reactions started with a 10-min denaturation at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 60°C, and 30 s at 72°C, and ended with an elongation step of 5 min at 72°C. Six microliters of PCR products was analyzed in a 2% NuSieve-agarose gel (FMC, Rockland, ME).

**Fluorescent labeling and adoptive transfer studies**

Three to five times restimulated cell lines were washed in PBS and labeled with CFSE (Molecular Probes) or PKH-26 (Sigma). For CFSE staining, cells were washed in serum-free DMEM and incubated in 2 nM CFSE/PBS for 20 min. The staining reaction was stopped by adding 10% horse serum in TCM. For PKH-26 labeling (25 \( \mu \)l of 2 × 104 M dye) was performed according to manufacturer’s protocol, and cell viability was determined by trypan blue (Sigma). A total of 100 \( \mu \)g/ml anti-\( \alpha \beta_1 \) mAb (clone GG5/3; mouse IgG1) or anti-\( \alpha \beta_2 \) mAb (clone WT-1; mouse IgG2A; PharMingen) was added to CFSE-labeled cell lines and incubated for 30 min at 37°C in 5% CO\(_2\). Cells were washed with TCM/5% serum and injected into LEW rats 1 day after kidney engraftment.

In some experiments, LEW\( _{w}^{\alpha} \) T lymphocytes (100 × 10\(^6\)) were infused i.v. into lightly \( \gamma \)-irradiated (450 rad) LEW rats that were then challenged 24 h later with cardiac allografts from BN donors. The immune potential of transferred cells can be accurately determined in such “test-tube” rat recipients (22).

**Tracing labeled cells by fluorescent microscopy and FACS analysis**

For histological analysis of PKH-26-labeled T cell distribution, organs were frozen in OCT. The 5-\( \mu \)m sections were mounted on gelatinized slides and dried for 60 min at room temperature. For fluorescence analysis, we used a laser microscope (Leica, Deerfield, IL). For flow-cytometric detection of fluorescent T cells, kidneys were removed, homogenized, and filtered through a 100-\( \mu \)m strainer. After washing twice in PBS, the pellet was resuspended with 10 ml DMEM (Life Technologies) containing 10 \( \mu \)g/ml collagenase type V (Sigma). Incubation was done at 37°C for 30 min. Leukocytes were isolated by Ficoll density separation (Life Technologies). For Ab staining, cells were incubated at 4°C for 20 min in 0.1 ml PBS with 1% bovine serum and mouse Cy-Chrome conjugated anti-rat CD4 mAb.
Arterial blood samples were lysed and fixed in 4% paraformaldehyde in PBS. Secondary staining was done using a PE-conjugated goat anti-mouse Ig (Zymed).

Results and Discussion

Generation and characterization of Ag-specific T cell lines

To determine the effects of alloantigen and integrin receptors on T cell homing, we generated LEW T cell lines with BN or WF allospecificity. Allospecificity was confirmed in a proliferation assay ([^3]H)Tdr incorporation) against syngeneic, allogeneic, or third-party γ-irradiated splenocytes. LEW<sub>BN</sub> T lymphocytes showed vigorous proliferation only after stimulation with BN, but not LEW or WF cells, whereas Lew<sub>WF</sub> T lymphocytes responded primarily against WF splenocytes (data not shown). The cell lines resembled a memory cell phenotype, with CD4<sup>+</sup>, CD8<sup>+</sup>, α<sub>4</sub>β<sub>1</sub>-integrin<sup>+</sup>, α<sub>5</sub>β<sub>2</sub>-integrin<sup>+</sup>, L-selectin<sup>low</sup>, and CD45 RC<sup>low</sup> (Fig. 1). Moreover, in vitro-generated cells showed type 1 effector phenotype, as evidenced by readily measurable CD4<sup>+</sup> proliferation during restimulations could be detected. By using RT-PCR analysis, all 22 known rat TCR V<sub>b</sub> usage despite clonal proliferation during restimulations could be detected. By using RT-PCR analysis, all 22 known rat TCR V<sub>b</sub> expression pattern after PCR amplification was not detectable even after PMA plus ionomycin stimulation (Fig. 1). No predominant Vβ usage despite clonal proliferation during restimulations could be detected. By using RT-PCR analysis, all 22 known rat TCR V<sub>b</sub> expression pattern after PCR amplification was not detectable even after PMA plus ionomycin stimulation (Fig. 1). No predominant Vβ usage despite clonal proliferation during restimulations could be detected. By using RT-PCR analysis, all 22 known rat TCR V<sub>b</sub> expression pattern after PCR amplification was not detectable even after PMA plus ionomycin stimulation (Fig. 1). No predominant Vβ usage despite clonal proliferation during restimulations could be detected. 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By using RT-PCR analysis, all 22 known rat TCR V<sub>b</sub> expression pattern after PCR amplification was not detectable even after PMA plus ionomycin stimulation (Fig. 1). No predominant Vβ The role of alloantigens on intragraft homing of Ag-activated T lymphocytes

The increased recruitment of LEW<sub>BN</sub> T lymphocytes to BN rather than LEW grafts may not necessarily be due to Ag-specific cell homing. Ongoing acute rejection could possibly amplify the inflammatory reaction in the allogeneic (BN) kidney. The attraction of more cells by highly inflamed endothelium could result in different infiltration pattern in allo/syngeneic grafts. To test our hypothesis that alloantigen is a critical factor in recruiting T lymphocytes to the graft, we generated a WF-specific cell line as a third-party control. A total of 25 × 10<sup>6</sup> CFSE-labeled LEW<sub>WF</sub> T cells and 25 × 10<sup>6</sup> PKH-26-labeled LEW<sub>BN</sub> T cells was simultaneously injected into LEW rats 24 h post-BN kidney engraftment. This protocol enabled us to evaluate the effects of alloantigens on intragraft homing of Ag-activated T lymphocytes.

Homing of donor Ag-specific T lymphocytes to organ allografts is a complex and highly regulated process (20, 23). It depends on the complementary interactions between endothelial cells, lymphocyte surface molecules, and local ECM proteins. Although most of ligand-receptor interactions appear to be redundant, some single factors carry critical nonredundant functions in the recruitment of certain lymphocyte subtypes toward inflamed tissues. The donor Ag is one of these mandatory factors in the process. It has been recently shown that the recognition of alloantigens presented by endothelial MHC class II complexes enhanced the transmigration of Ag-specific lymphocytes through endothelial cell layers in vitro (24). Interestingly, the inflammatory response due to ischemia/reperfusion injury after transplantation has led to up-regulation of MHC class II molecules on the endothelial surface (25).

In this study, the distribution of alloreactive T lymphocytes was evaluated at different time points. One day after transplantation, 15 × 10<sup>6</sup> PKH-26-labeled cells were injected into groups of allogeneic (BN to LEW) and syngeneic (LEW to LEW) kidney-grafted rats. We used cells from the same lines and batches for each group of syngeneic and allogeneic transplanted rats. This allowed the quantification of differences due to graft-related effects. Several organs were harvested 12, 24, and 48 h after cell transfer, and the distribution of fluorescence-stained lymphocytes was monitored by counting 5-μm tissue sections (Fig. 1). Early observation of fluorescent light emission of PKH-26 did not allow us to distinguish between labeled cells and autofluorescence background at later than 48 h. The highest number of labeled cells was found throughout in recipients' lung and liver. LEW<sub>BN</sub> T lymphocytes do recirculate through secondary lymphatic organs, as shown by the appearance of labeled cells in peripheral LNs and spleens. No T cell homing to contralateral nontransplanted kidneys was found.

An increased recruitment of labeled T cells was detectable only in the allograft, whereas the number of cells infiltrating syngeneic kidney grafts and other organs remained stable.

The earliest timepoints studied and increased with time probably due to the intragraft proliferation, apoptosis of donor-reactive T cells outside the transplant, and/or trapping of recirculating cells, perhaps by direct allorecognition, inside the graft.

Distribution of PKH-26-labeled LEW<sub>BN</sub> T lymphocytes after adoptive transfer

Homing of donor Ag-specific T lymphocytes to organ allografts is a complex and highly regulated process (20, 23). It depends on the
graft) were harvested 24 h after the transfer. Isolated graft-infiltrating cells were stained with anti-rat CD4 mAb, and FACS analysis was performed. Dot blots representing the ratio of green-labeled WF- to red-labeled BN-specific cells in lung and in the transplanted kidney are shown in Fig. 4. The ratio of injected cells measured before injection was found to be identical with the ratio in lung or liver. In contrast, a 40% increase in numbers of BN-specific lymphocytes as compared with WF-specific cells was detected in the BN graft. We also injected a mixture of equal numbers of PKH-26- and CFSE-labeled LEW<sub>BN</sub> T cells into LEW recipients of BN renal grafts. These controls did not show any significant changes in the red to green ratio in all tested organs, implying that alloantigen itself may represent a factor in recruiting the lymphocytes to the graft. The impact of alloantigen can be explained by three not mutually elusive principles. First, alloantigen-specific lymphocytes home into the graft in an Ag-specific manner (24). High MHC class II expression following transplantation might initiate the cross-talk between graft endothelium and

**FIGURE 2.** PKH-26-labeled LEW<sub>BN</sub> T lymphocytes in vivo. The digital images of PKH-26-labeled (red) cells in various tissues 48 h after adoptive T cell transfer in rat renal allograft recipients. A, Allogeneic transplanted kidney tubular region; tubulus emitted yellow autofluorescence signal (original magnification, ×100). B, allogeneic transplanted kidney glomerula region (original magnification, ×100). C, lung (original magnification, ×40). D, liver (original magnification, ×40). Representative of four distinct experiments.
adoptively transferred CD4$^+$ T lymphocytes (25). The donor-devised endothelial cells present the alloantigen via the direct pathway. The protocol, used to generate allospecific cell lines, promotes this direct interaction because TCR activation during in vitro priming and expansion was performed by donor (BN)-derived MHC class II molecules. Second, clonal expansion inside the graft or in secondary lymphatic organs may change the total number of alloreactive T lymphocytes. Third, random homing of activated T cells to organ grafts was followed by enhanced apoptosis in the absence of alloantigens (26).

**Pretreatment with anti-$\alpha_4\beta_1$ but not anti-$\alpha_4\beta_2$ mAb inhibits intragraft homing of Ag-specific T cells**

Ag-activated and naive T lymphocytes exhibit distinct in vivo recirculation and homing patterns (3, 20, 23). Although these differences may not be absolute, activated cells have a higher potential to transmigrate and accumulate at local inflamed tissues, whereas naive T lymphocytes circulate between peripheral lymphatic compartments. This may be due, at least in part, upon the expression of different homing receptors on their surface. The $\alpha_4\beta_1$, a member of the $\beta_1$ integrin family, and $\alpha_4\beta_2$, a $\beta_2$ integrin, are both highly expressed on activated, but not quiescent T lymphocytes (13). It has been shown that T cell activation dramatically enhances the binding affinity between $\alpha_4\beta_1$ and its endothelial counterreceptor VCAM-1 (16). These features support the role of $\alpha_4\beta_1$ and $\alpha_4\beta_2$ as potential targets for therapeutic intervention in transplant recipients. Indeed, we have recently shown that targeting $\alpha_4\beta_1$ in vivo interactions significantly prolongs the survival of cardiac allografts in rats (27). Although other studies showed only marginal effects of anti-$\alpha_4\beta_1$ or anti-$\alpha_4\beta_2$ mAb monotherapy, cotreatment with both Abs often resulted in an indefinite survival of murine heart and islet grafts (28, 29).

We have then attempted to evaluate the role of $\alpha_4\beta_1$ and $\alpha_4\beta_2$ upon the homing of activated alloreactive CD4 T lymphocytes in allograft recipients. CFSE-labeled LEWBN T lymphocytes were incubated with anti-$\alpha_4\beta_1$ or anti-$\alpha_4\beta_2$ mAb to block interactions with their counterreceptors. Preincubation had no influence on $\alpha_4\beta_1$ or $\alpha_4\beta_2$ surface receptor density, as shown by comparing direct staining of control lymphocytes and secondary staining of preincubated cells. After removing unbound Ab by extensive washing, $25 \times 10^6$ mAb-treated CFSE$^+$ cells were injected in concert with an equal number of nontreated PKH-26-labeled LEWBN T lymphocytes into rat recipients of renal allografts. We did not mix both populations before injection to avoid binding of contaminating Ab to nontreated PKH-26 control cells. Inhibition/augmentation of cell sequestration was calculated by defining the CFSE/PKH-26 ratio in lungs as the injection ratio. The organs were harvested 24 h after the transfer, and graft-infiltrating cells were isolated/stained, as described above. Although we were able to detect labeled cells in spleen, peripheral LNs, and blood, the percentage in comparison with hosts native CD4 lymphocytes was too low (<0.05%) to quantify distribution differences after 24 h. Fig. 5 shows the distribution of pretreated and untreated labeled cells in rat kidney allografts. Ex vivo treatment with anti-$\alpha_4\beta_1$

**FIGURE 3.** Distribution kinetics of LEWBN T lymphocytes in rat renal transplant recipients. A total of $15 \times 10^6$ of LEWBN T lymphocytes was injected into syngeneic and allogeneic transplanted rats. Tissues were harvested 12, 24, and 48 h after adoptive transfer, and fluorescent cells were counted on tissue sections. Data are expressed as ratio between cells in allogeneic and syngeneic transplanted rats ($n = 3$).
mAb inhibited infiltration of renal allografts with CFSE-labeled mAbs by 85% (p < 0.05). In contrast, no significant inhibition of anti-αβ2 mAb-treated cells was detectable. The intraluminal and liver ratios independent of any kind of treatment were nearly identical in all treatment groups. In addition, adoptive transfer studies with two dyes andwithout any Abs showed no different homing behavior into the graft or into other organs (e.g., lung), implying that the two dyes do not appear to differentially affect cell migration patterns. Moreover, the difference in graft infiltration after αβ1, and αβ2 mAb treatment was not due to different mAb densities on cell surfaces, or isotype-dependent clearance of mAb-coated cells. Indeed, equal numbers of treated and untreated cells sequestered in hosts’ spleens and livers. Both mAb preparations had no effect on the surface phenotype of transferred T cells, their activation (CD25 expression), proliferation ([3H]Thymidine incorporation), or isotype-dependent clearance of mAb-bearing cells (data not shown).

The limited number of circulating adoptively transferred cells at later time points (<0.02% after 24 h) favors the hypothesis that the first hours after leaving of lymphoid organs are most critical for selective trafficking of activated allogeneic lymphocytes into the allograft. During this early time frame, anti-αβ1 mAb was detectable on the cell surface (data not shown), suggesting a direct interfering in the rolling and transmigration process inside the donor graft as causal mechanism of the blocked intragraft mononuclear cell infiltration. On the other hand, the beneficial effects of anti-αβ1 mAb therapy in some transplantation models (28, 29) may not be due to diminished intragraft recruitment of activated alloreactive CD4+ cells. Perhaps direct signaling effects through LFA-1 on VCAM-1. Nevertheless, anti-αβ1 mAb pretreatment (n = 3); anti-αβ2 mAb pretreatment (n = 4).

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