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Abrogation of Functional Selectin-Ligand Expression Reduces Migration of Pathogenic CD8⁺ T Cells into Heart

Yi Hong Cai,* Angeles Alvarez,* Pilar Alcaide,* Paurene Duramad,* Yaw-Chin Lim,‡ Petr Jarolim,† John B. Lowe,*§ Francis W. Luscinskas,* and Andrew H. Lichtman²*

CD8⁺ T cells are involved in autoimmune and infectious myocarditis and cardiac allograft rejection. The role of selectins in cardiac recruitment of CD8⁺ T cells is not understood. In this study, the contribution of T cell selectin ligands to effector CD8⁺ T cell recruitment into the heart was examined using a model of myocarditis, which depends on transfer of OVA peptide-specific CD8⁺ T cells (OT-I) into mice (CMy-mOva) that express OVA in the heart. α-(1,3)-Fucosyltransferase (FucT)-VII-deficient OT-I cells displayed over a 95% reduction in their ability to interact with P-selectin under flow conditions in vitro, compared with wild-type OT-I cells. Interaction of FucT-VII-deficient OT-I cells with E-selectin was reduced ~50%. FucT-VII-deficient OT-I cells were also less efficiently recruited into a dermal site of Ag and adjuvant injection. Significantly, FucT-VII-deficient OT-I cells were also impaired in their ability to migrate into CMy-mOva hearts, compared with wild-type OT-I cells. Transfer of FucT-VII-deficient T cells caused less severe early myocarditis and myocardite damage than transfer of wild-type T cells. Combined FucT-IV/VII-deficient OT-I cells displayed a more profound reduction in E-selectin interactions in vitro compared with FucT-VII-deficient T cells, and the FucT-IV/VII-deficient T cells also showed less early recruitment and pathogenicity in the CMy-mOva myocarditis model. These results identify a prominent role for selectin ligands in contributing to effector CD8⁺ T cell recruitment into the myocardium and indicate that selectin-dependent T cell recruitment is relevant to other tissues besides the skin. The Journal of Immunology, 2006, 176: 6568–6575.

Effect CD8⁺ T cells contribute significantly to the pathogenesis of infectious myocarditis, autoimmune myocarditis, and cardiac allograft rejection (1). Nonetheless, the mechanisms by which these T cells are recruited to the myocardium are incompletely understood. Most studies of lymphocyte trafficking into tissues have focused on CD4⁺ T cells, and few of these studies have addressed trafficking into heart. A better understanding of CD8⁺ T cell traffic into the heart is important for design of therapeutic strategies to reduce severity of T cell-mediated myocardial disease.

The differentiation of effector T cells from naive precursors after Ag recognition results in the acquisition of effector functionality as well as a change in migratory phenotype. In particular, effector T cells express chemokine receptors and adhesion molecules not expressed by naive T cells, and these molecules facilitate recruitment into peripheral tissues, especially at sites of inflammation. One important component of the migratory phenotype of effector T cells is the expression of functional glycoprotein ligands that bind to E- and P-selectin on endothelial cells (2). The synthesis of these ligands in T cells, as well as other leukocytes, is dependent on posttranslational modification by α-(1,3)-fucosyltransferase (FucT)-IV and FucT-VII, tyrosyl protein sulfotransferases (TPST-1 and TPST-2), and C2GlCNAC-T-I (core 2 β-1,6-glucosaminyltransferase-I). The expression of these enzymes during effector T cell differentiation is regulated by signals generated by cytokine and T cell Ag receptors. In both CD4⁺ and CD8⁺ T cells, FucT-VII is the major fucosyltransferase required for E- and P-selectin ligand synthesis, but FucT-IV may be required for a small fraction of T cell selectin-binding activity (3, 4).

The analysis of the role of selectin-selectin ligand interactions in effector T cell trafficking in vivo is complicated by the fact that L-selectin, expressed on naive T cells, and L-selectin ligands expressed by the high endothelial venules of secondary lymphoid organs, are required for trafficking of these cells into lymph nodes where T cell responses are initiated and effector T cells are generated. Therefore, abrogation of selectin ligand synthesis by fucosyltransferase gene ablation will impair L-selectin ligand synthesis by high endothelial venules, which will impair naive T cell homing to lymph nodes and the generation of effector T cells. Alternatively, T cell trafficking can be studied by adoptive transfer of normal effector T cells into mice in which E- and/or P-selectin are blocked with Abs or into mice with genetic deficiencies in selectin expression. This approach, however, has the limitation that secondary inflammatory events involving recruitment of endogenous leukocytes will be inhibited. Secondary inflammation can enhance selectin-independent T cell recruitment.

To facilitate the analysis of migration and effector function of a monospecific population of CD8⁺ T cells that recognize an Ag expressed exclusively in the heart, we have developed a transgenic model of CD8⁺ T cell-mediated myocarditis (5). This model uses

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*Vascular Research Division and †Laboratory Medicine Division, Department of Pathology, Brigham and Women’s Hospital and Harvard Medical School, Boston, MA 02115; ‡Department of Pathology and Physiology and Immunology Program, Faculty of Medicine, National University of Singapore, Singapore; and §Department of Pathology, Case Western Reserve University School of Medicine, Cleveland, OH 44106

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2 Address correspondence and reprint requests to Dr. Andrew H. Lichtman, Department of Pathology, Brigham and Women’s Hospital, 77 Avenue Louis Pasteur, New Research Building 752N, Boston, MA 02115. E-mail address: alichtman@rics.bwh.harvard.edu

3 Abbreviations used in this paper: FucT, α-(1,3)-fucosyltransferase; TnI, troponin I.
CMy-mOva mice that express OVA under the control of the cardiac myosin H chain promoter, and OT-I cells, which are TCR-transgenic CD8+ T cells specific for an OVA peptide bound to the class I MHC molecule H-2Kk. Within 4–6 days of adoptive transfer into CMy-mOva mice, effector OT-I cells migrate into the heart and cause a myocarditis. When this model is used with mutant OT-I cells, it affords the opportunity to assess the contributions of specific genetic loci to trafficking and posttrafficking functions that contribute to myocarditis. In the present study, we examined the specific genetic loci to trafficking and posttrafficking functions that determine myocarditis.

Materials and Methods

Mice

All mice used in the current study were bred in the pathogen-free facility at the Eugene Braunwald Medical Research Center or the Harvard Medical School Warren Alpert Building, in accordance with the guidelines of the committee of Animal research at the Harvard Medical School and the National Institutes of Health animal research guidelines. The CMy-mOva transgenic mouse line that expresses OVA in the heart (5) was carried on both C57BL/6-Thy1.2 (CD90.2) and Thy1.1 (CD90.1) backgrounds, and all experimental animals were heterozygous for the OVA transgene. The OT-I TCR-transgenic mouse strain (6) was provided by W. R. Heath and F. Carbone (Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia) and was maintained on a C57BL/6-Thy1.2 (CD90.2) background. The OT-I TCR is expressed on CD8+ T cells and is specific for the OVA peptide 257–264 (SIINFEKL) bound to the class I MHC molecule H2-Kk (7). Previously derived FucT-VII- and FucT-IV/- mice on a C57BL/6 background (8), were cross-bred with OT-I mice to generate OT-I+/FucT-VII/- and OT-I+/FucT-IV/-. FucT-VII- and FucT-IV/- mice are heterozygous for the OT-I TCR transgene, and naive OT-I cells were isolated from the suspensions by CD8 magnetic beads (Miltenyi Biotec), as described previously (5, 9). The naive T cells were stimulated in vitro with mitomycin C-treated (Sigma-Aldrich) C57BL/6 spleen cells and SIINFEKL (1 μg/ml) at a final concentration of 1 μg/ml/L. These cultures were supplemented with 2 μg/ml anti-CD28 (clone 37.51; BD Pharmingen), 50 μ/ml recombinant mouse IL-2 (R&D Systems), and 10 ng/ml recombinant mouse IL-12 (R&D Systems). The cultures were placed in 75-cm2 flasks and incubated at 37°C, 5% CO2. After 3 days of stimulation, all cultures were diluted 1/1 with fresh medium containing 20% FCS (Sigma-Aldrich) and 2 mmol/L sodium pyruvate, 100 μM penicillin, 100 μg/ml streptomycin, 1 μg/ml gentamycin (Invitrogen Life Technologies), 5% FBS (Laboratories), and recombinant mouse IL-2 (R&D Systems) at various E:T cell ratios. Target cells were pulsed with 51Cr sodium chromate-loaded H-2Kk-expressing EL4 target cells (American Type Culture Collection) at various E:T cell ratios. Target cells were pulsed with 51Cr (R&D Systems) were captured on glass coverslips using goat F(ab’)2 anti-humanFcAb as previously detailed (12), and the coverslips were placed in a parallel plate flow chamber. Effector T cells were resuspended in Dulbecco's PBS containing 0.1% human serum albumin and 20 mM HEPES (pH 7.4), at 37°C (5 × 106 cells/ml) and were drawn through the flow chamber at decreasing flow rates for 2.5 min each, i.e., 0.94 ml/min (estimated shear stress = 1.5 dynes/cm2), 0.78 ml/min (1.0 dynes/cm2), 0.52 ml/min (0.7 dynes/cm2), and 0.26 ml/min (0.4 dynes/cm2). T cell interactions with immobilized selectins were recorded using a ×20 phase contrast objective and a PC-based videomicroscopy system (Videolab Software; Ed Marcus Laboratories). T cell accumulation was determined after the final minute of each flow rate by counting the number of interacting cells in four different fields. Specificity of binding was confirmed by adding blocking Abs to the perfusate. For this purpose, we used anti-E-selectin Ab (9A9) and anti-P-selectin Ab (RB40.34), both generously provided by Dr. K. Ley (University of Virginia, Charlottesville, VA), at final concentrations of 10 μg/ml.

In vitro analysis of T cell functional phenotype

Wild-type or FucT-VII-deficient effector OT-I cells were removed from primary activation cultures at day 5, washed and resuspended in RPMI (1640 medium, and used for functional assays. Cytotoxic activity of effector OT-I cells was measured by coculturing the T cells with 51Cr (Sigma-Aldrich) C57BL/6 spleen cells and SIINFEKL (1 μg/ml) in 200-μl round-bottom wells. Culture supernatants were harvested at 48 h and IFN-γ was detected by ELISA, as described previously (5).

Serum troponin I (Tnl) determination

Blood was collected from mice at time of sacrifice, and serum fractions were isolated and transferred frozen to the Clinical Chemistry Laboratory at the Brigham and Women’s Hospital (Boston, MA). Mouse cardiac Tnl was measured using the ADVIA Centaur cTnl assay (Bayer). This automated sandwich immunoassay uses a primary polyclonal goat anti-TnI Ab and a secondary combination of monoclonal mouse anti-Tn Ab. The assay range is from 0.10 to 50 μg/L, sample volume is 100 μl, and total imprecision of 3.6% at 43.62 μg/L and 6.6% at 0.94 μg/L. As indicated below,
we found that the ADVIA Centaur human cTnI immunoassay can detect mouse TnI. To verify the utility of this method for a semiquantitative assessment of myocardial damage in mice, we performed serial (up to 100 times) dilutions of mouse specimens with high TnI levels and detected a proportional decrease in signal with decreasing mouse TnI concentrations.

**Flow cytometry analyses**

All cell preparations were washed twice in staining buffer (Dulbecco’s PBS with 1% BSA). For phenotypic analysis of surface markers, 0.5 × 10^6 cells were suspended in 100 µl of staining buffer containing 1 µg each of specific Ab or matched species/isotype control Ig, purchased from BD Pharmingen, and incubated on ice for 20 min followed by washing and fixation with 0.5% paraformaldehyde. Stained cell preparations were then analyzed by flow cytometry using a FACSCalibur instrument and CellQuest software (BD Biosciences). Abs used for flow cytometry were as follows: PE-conjugated anti-mouse CD90.1/Thy1.1 (clone OX-7); FITC-conjugated anti-mouse CD44 (clone IM7); FITC-conjugated anti-mouse CD25 (IL-2R α-chain) (clone 7D4); FITC-conjugated LFA-1 (clone M174); PE-conjugated L-selectin (ME14); FITC-conjugated anti-P-selectin glycoprotein ligand-1/CD162 (clone 2PH1), and FITC-conjugated anti-VLA4 (clone 9C10).

**Statistical analysis**

Statistical analyses were performed using the Mann-Whitney U test for data that was not normally distributed, as determined by the Kruskal-Wallis test, and the Students t test for normally distributed data. A value of p < 0.05 was considered to be significant.

**Results**

**FucT-VII−/− deficiency does not impair activation or effector function of CD8+ T cell in vitro**

The number of CD8+ T cells isolated from lymph nodes and spleen of FucT-VII−/− OT-I mice were roughly equivalent to the yields from wild-type OT-I mice. This finding is in contrast to a decrease in peripheral node CD8+ T cells recovered from peripheral nodes of non-TCR-transgenic FucT-VII−/− mice, as reported previously (4). This apparent discrepancy can be explained by the fact that in the present study, most of the CD8+ T cells are derived from the spleen, and there is no evidence that selectin ligands are required for naive T cell migration into the spleen (13). The number of FucT-VII−/− and wild-type OT-I cells recovered after 5 days of Ag stimulation in vitro, was also approximately the same. Analysis of the expression of CD25, CD44, and L-selectin by FACS did not differ significantly between the FucT-VII−/− and wild-type OT-I cells recovered 5 days after Ag stimulation in vitro (data not shown). These findings indicate FucT-VII-deficient T cells retain a normal capacity to be activated by Ag and to undergo proliferative responses. FucT-VII−/− and wild-type OT-I cells expressed equivalent levels of LFA-1, VLA-4, and P-selectin glycoprotein ligand-1 (data not shown).

FucT-VII-deficient did not influence the effector function of activated OT-I cells. Cytotoxic function of FucT-VII−/− and wild-type OT-I cells were equivalent (Fig. 1A). The amount of IFN-γ produced by Ag activation of FucT-VII−/− and wild-type OT-I cells was not significantly different (Fig. 1B).

**FucT-VII deficiency reduces CD8+ T cell interactions with E- and P-selectin**

The relative abilities of wild-type and FucT-VII-deficient T cells to interact with selectins was compared using a flow chamber assay and immobilized recombinant P- and E-selectin. FucT-VII-deficient OT-I cell binding to P-selectin was negligible at all shear stresses tested, whereas wild-type OT-I cell binding was readily detected (Fig. 2A). FucT-VII deficiency resulted in an ∼50% reduction in OT-I cell binding to E-selectin, compared with wild-type OT-I cells (Fig. 2B). The residual binding of FucT-VII-deficient OT-I cells was eliminated by a function blocking mAb to E-selectin (data not shown), demonstrating the specificity of adhesion. The residual E-selectin adhesion could be due to a contribution from FucT-IV because FucT-IV/VII-deficient OT-I cells do not bind E-selectin under shear flow conditions (shown in later experiment). FucT-VII deficiency did not influence binding of OT-I cells to VCAM-1 under identical flow conditions (Fig. 2C). These results indicate that the binding of FucT-VII-deficient T cells to E-selectin is significantly reduced, relative to wild-type cells, whereas binding to P-selectin is nearly abolished. As a control, FucT-VII-deficient T cell adhesion to VCAM-1, a VLA-4-dependent interaction, was robust and similar to that of wild-type OT-I cells.

**FucT-VII deficiency reduces CD8+ T cell migration into the skin**

FucT-VII-dependent selectin ligand expression is required for both CD4+ and CD8+ T cell homing to skin (4, 8, 14). We used a footpad immunization protocol to ensure that the FucT-VII-deficient OT-I cells are also defective in selectin-dependent homing in vivo. Significantly more adoptively transferred wild-type OT-I cells were present in OVA-immunized footpads compared with footpads injected with CFA alone (Fig. 3), indicating that the presence of the cognate Ag in the tissues led to enhanced recruitment and/or enhanced local proliferation of the T cells. However, there were significantly fewer FucT-VII-deficient OT-I cells than wild-type OT-I cells in sections of footpad 3 days after immunization with OVA (Fig. 3). These results are consistent with impaired migration of the FucT-VII-deficient T cells into the skin.

**FucT-VII deficiency reduces the ability of adoptively transferred CD8+ T cells to cause myocarditis**

We then asked whether T cell interactions with endothelial selectins contribute to entry of effector CD8+ T cells into the heart in the CMy-mOva model of myocarditis, and thereby contribute to myocardial histopathology. We examined histological sections of CMy-mOva hearts after adoptive transfer of OT-I effectors, and we scored the degree of myocarditis. The mean histological myocarditis score at 96 h after transfer of 2.5 × 10^6 FucT-VII-deficient OT-I cells was 56% lower than after transfer of the same number of wild-type OT-I cells (Fig. 4A). Because the recipient CMy-mOva mice have no ongoing inflammatory processes in the heart at the time of T cell transfer, we reasoned that selectin ligand expression is not likely to influence the earliest recruitment of T cells. To better discern differences in the migration of selectin

**FIGURE 1.** Equivalent effector phenotype of FucT-VII-deficient and wild-type CD8+ T cells. A. CTL activity against SIINFEKL (Ag)-pulsed and unpulsed EL4 target cells was measured by 51Cr release. The data are from one experiment of two performed with similar results. B. IFN-γ secretion by Ag-stimulated FucT-VII-deficient and wild-type CD8+ T cells was measured by ELISA after 48 h. The data represent mean ± SD of triplicate determinations from one experiment of three performed with similar results.
ligand-deficient and wild-type OT-I cells into the heart, we pretreated mice with LPS (75 ng/gm) i.p. 4 h before transfer of T cells. The mean histological myocarditis score 96 h after transfer of 2.5 $\times$ 10^6 wild-type OT-I cells into LPS-treated CMy-mOva mice was 184% higher than the mean histological score after transfer of the same number of cells into untreated CMy-mOva mice (Fig. 4), indicating that LPS treatment did enhance the OT-I-induced inflammatory response in the heart. The histological score after transfer of FucT-VII-deficient OT-I cells into LPS-pretreated CMy-mOva mice was 46% lower than after transfer of the same number of wild-type OT-I cells into LPS-treated animals (Fig. 4B). Thus the FucT-VII dependency of the OT-I-mediated inflammatory response was moderately reduced in the presence of LPS pretreatment. The reduced pathogenicity of FucT-VII-deficient T cells suggests that selectin ligands are expressed on heart endothelial cells during OT-I-mediated myocarditis. We confirmed this to be the case by immunohistochemical detection of E-selectin in heart sections from mice with ongoing OT-I-mediated myocarditis (Fig. 5). We could not detect E- and/or P-selectin expression by immunohistochemistry in hearts of CMy-mOva mice that did not receive OT-I transfers (data not shown).

**FIGURE 3.** Reduced migration of FucT-VII-deficient CD8⁺ T cells into dermal site of injection of Ag and adjuvant. Thy1.2⁻⁺/⁻ C57BL/6 mice were injected in one foot pad with CFA with our without OVA (50 μg/mouse), followed 5 h later by i.p. injection of 5 $\times$ 10^6 wild-type (WT) or FucT-VII-deficient (FucT-VII KO) OT-I Thy1.1⁺⁺ T cells. Three sections were examined per mouse, and three mice were included in each group. Each data point represents the mean number of Thy1.1⁺⁺ T cells per footpad section in each mouse. The horizontal lines represent the medians of each group. The differences in medians of OVA-treated wild-type and FucT-VII-deficient mice were significant ($p < 0.05$).

**FIGURE 2.** Impaired interaction of FucT-VII-deficient CD8⁺ T cells with E- and P-selectin under flow. The data represent the accumulation of wild-type (WT) and FucT-VII-deficient (FucT-VII KO) T cells on glass coverslips coated with recombinant P-selectin Ig (A), E-selectin Ig (B) or VCAM-1 (C) under the indicated shear stresses. The data represent the mean ± SD values from three separate experiments, with triplicate determinations in each experiment.

**FucT-VII deficiency impairs the ability of adoptively transferred CD8⁺ T cells to enter the myocardium**

Although the histological analysis of myocarditis is consistent with reduced migration of FucT-VII OT-I cells into the heart compared with wild-type T cells, the histological score reflects the degree of inflammatory infiltrate, irrespective of cell type, including endogenous T cells and neutrophils. The degree of secondary inflammation that may occur in response to OT-I infiltration may not be linearly related to the number of T cells that enter the heart, and therefore differences in myocarditis score may not be a sensitive indicator of differences in T cell migration. To more specifically assess whether FucT-VII deficiency results in less migration of OT-I cells into the heart, we adoptively transferred Thy1.2 (CD90.2) expressing wild-type or FucT-VII-deficient OT-I cells into Thy1.1 (CD90.1) homozygous CMy-mOva mice, and used immunohistochemistry to quantify the number of Thy1.2 OT-I cells in myocardial sections at 96 h after transfer. This time point was chosen because...
previous work with our model indicates that transferred wild-type OT-I effectors are first detectable in a mediastinal draining lymph node, but they have left this node and are readily detectable in the heart by 96 h (5). The analysis revealed that FucT-VII deficiency caused a 98% reduction in T cell migration into the heart when 1.0 x 10^6 T cells were transferred, and an 88% reduction when 2.5 x 10^6 T cells were transferred (Fig. 6).

**Impaired migration of FucT-VII-deficient T cells into the heart results in reduced myocyte damage**

The data discussed so far indicate there is still a significant histologically identifiable inflammatory response in the CMy-mOva hearts after selectin ligand-deficient CD8^+ T cell transfer, even though the actual number of these T cells that enter the heart is only a small percent of the number of normal CD8^+ T cells that enter the heart under identical conditions. OT-I effector T cells have direct cytotoxic activity against OVA expressing cells, such as the cardiac myocytes in CMy-mOva mice. We decided to measure myocyte damage as another comparative indicator of the ability of selectin ligand-deficient and normal T cells to leave the circulation, enter myocardium and thereby promote myocardial pathology. Previously we have shown that serum levels of cardiac TnT and TnI are a sensitive and specific indicator of myocyte damage in the CMy-mOva model (5). In this study, we adapted a clinical laboratory assay for cardiac TnT to follow mouse heart damage. Significantly elevated TnT levels were detectable 96 h after T cell transfer of normal OT-I cells into CMY-mOva mice, and the levels were 88% reduced in mice receiving FucT-VII-deficient T cells (Fig. 7). These data are consistent with the immunohistochemical data, and indicate that functional selectin ligands play an important role in the migration of the effector CD8^+ T cells into myocardium.

**Reduced pathogenicity of FucT-IV/VII-deficient T cells**

The residual capacity of FucT-IV/VII-deficient OT-I cells to enter the heart and cause myocarditis may reflect selectin-independent migration and/or residual selectin ligand synthesis dependent on FucT-IV. Although previous studies have reported that only a small percentage of the normal amount selectin binding activity is retained in T cells from FucT-VII null mice, our in vitro flow studies with FucT-VII-deficient OT-I cells (see Fig. 2) indicate significant residual capacity to bind E-selectin. To address this issue, we performed experiments with combined FucT-IV/VII^−/− OT-I mice. These mice are more difficult to breed, limiting the number of experiments that could be performed. In vitro flow chamber experiments indicated that almost all P- and E-selectin binding activity seen in normal OT-I effector cells was lost in FucT-IV/VII^−/− OT-I effector cells (Fig. 8). The almost complete loss of E-selectin binding by FucT-IV/VII^−/− OT-I effector cells is in contrast to the significant residual binding by FucT-VII^−/− OT-I effector cells, shown in Fig. 2. However, the pathologic consequences of adoptive transfer of FucT-IV/VII^−/− OT-I cell into CMY-mOva mice were similar to those described above after transfer of FucT-VII^−/− OT-I. In particular, the mean myocardial inflammation score after FucT-IV/VII^−/− OT-I transfer was ~50% less than after wild-type OT-I transfer (Fig. 9A). Serum TnI levels after FucT-IV/VII^−/− OT-I transfer were 84% less than after transfer
of wild-type OT-I cells (although the difference was not statistically significant). These results confirm the important role that fucosyltransferase-dependent selectin ligand synthesis plays in migration of CD8$^+$T1 cells. However, they do not support the hypothesis that FucT-IV expression accounts for residual cardiac migratory capacity seen in the FucT-VII-deficient CD8$^+$T1 cells. A caveat in the interpretation of these results is the limited number of mice studied, leaving the possibility that the double knockout OT-I cells may be less pathogenic than the single knockout cells.

Discussion
In this study, we have observed that functional selectin ligands contribute significantly to the migration of cardiac Ag specific CD8$^+$ effector T cells into the heart. In particular, abrogation of fucosyltransferase-dependent E- and P-selectin binding activity markedly reduced the capacity of CD8$^+$ effector T cells to enter the heart and cause myocyte damage. Our model of CD8$^+$ T cell-mediated myocarditis was developed to study migration and effector functions of T cells specific for an Ag expressed exclusively in the heart, and is therefore relevant to the pathogenesis of cardiac allograft rejection and both viral and autoimmune myocarditis. Although E-selectin likely plays an important role in the acute inflammatory response associated with ischemia reperfusion injury in the heart (15), there is little published information about endothelial selectins in T cell-mediated cardiac inflammation. E-selectin is reported to be up-regulated in murine T cell mediated myocarditis (16). FucT-VII deficiency in murine recipients of cardiac allograft prolonged survival of the grafts (17), and a selectin ligand mimic has been shown to reduce the inflammatory response in rat cardiac allograft (18). These studies did not distinguish the importance of T cell selectin ligands from the ligands on other leukocytes. We are not aware of any previous studies specifically addressing selectin-dependent migration of CD8$^+$ T cells into the heart.

Previous work has clearly demonstrated that endothelial selectins and/or their leukocyte ligands are required for the migration of effector CD4$^+$ and CD8$^+$ T cells of the Th1/Tc1 phenotype into dermal inflammatory sites (19–25) and experimentally induced peritonitis (26). However, the importance of endothelial selectins and their ligands for CD4$^+$ and CD8$^+$ T cell migration into tissues other than skin has been questioned. For example, CD4$^+$ T cell-dependent inflammatory response in
the brain in experimental autoimmune encephalitis appears intact in the absence of endothelial selectins (27). Furthermore, CD8+ T cell-dependent clearance of lymphocytic choriomeningitis virus from several tissues, including liver, lung, and spleen is not impaired in E-/P-selectin null mice (28). Similarly, CD8+-dependent clearance of lymphocytic choriomeningitis virus or vesicular stomatitis virus from several organs, including brain, ovary, liver, lung, and spleen, was equivalent in FucT-VII-deficient and wild-type mice (14). Our results do not contradict these previous studies because they did not address cardiac inflammation. We believe that regulation of inflammatory responses in the heart likely differs from other tissues, especially those tissues in which exposure to environmental pathogens is a normal and frequent occurrence (29). Furthermore, our disease model, in which effector T cells are transferred into quiescent CMV-mOva mice, differs significantly from the situation of a systemic viral infection, in which strong innate immune responses with accompanying inflammation are likely to generate multiple, redundant molecular signals for T cell recruitment.

Although our data demonstrate selectin-dependent migration of T cells into the heart, it is unclear whether the earliest T cell migration into the myocardium is selectin-dependent in our model. Morphologic and gene expression analyses indicate that in the absence of OT-I transfer, CMV-mOva hearts are not inflamed (5, 9), and endothelial selectin expression is not detectable by immunohistochemistry before OT-I transfer. Nonetheless, early T cell immigrants rapidly trigger an inflammatory response. Induced endothelial selectin expression clearly enhances the recruitment of OT-I cells, as they are likely to enhance recruitment of endogenous leukocytes, including neutrophils. It remains possible that the uninnflamed myocardial microvasculature may express low basal levels of endothelial E- and P-selectin, which are below the detection limit of immunohistochemistry, but nonetheless contribute to early T cell recruitment. Basal expression of E-selectin has been detected in cardiac microvessels (30), and this reflects the importance of the skin in immune surveillance. In contrast, immune surveillance is not likely to be an adaptive function that has evolved in the heart (29), and therefore constitutive expression of selectins on myocardial microvascular endothelial cells would not be expected.

Previous studies suggest that L-selectin ligand on endothelial cells mediates recruitment of T cells into human and rat cardiac allografts (31, 32). In our adoptive transfer model, FucT-VII deficiency is limited to the transferred T cells, and L-selectin ligand expression by endothelial cells is therefore not altered. Because we see marked reduction of FucT-VII-deficient OT-I cells into heart and skin, it appears that these T cells rely more on endothelial selectins for recruitment rather than on endothelial L-selectin ligand binding to T cell L-selectin. In fact, there is little L-selectin expression on the effector OT-I cells that we transfer. It is possible that the kinetics of clearance of the adoptively transferred T cells from the circulation, relative to the timing of endothelial L-selectin expression, in our model does not recapitulate what happens during a T cell response that is endogenously initiated.

In summary, our data demonstrate that effector CD8+ T cells specific for a cardiac Ag use selectin ligand interactions with cardiac endothelial selectins as part of the process of migration into myocardium. The requirement of selectin-selectin ligand interaction is likely to limit potentially dangerous entry of activated T cells into uninfamed myocardium. Based on these results, it is reasonable to consider selectin blockade for the prevention of T-cell-mediated cardiac inflammatory processes, such as allograft rejection.

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