Targeting CD45RB alters T cell migration and delays viral clearance

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Abstract

CD45 is a receptor tyrosine phosphatase essential for TCR signaling. One isoform, CD45RB, is down-regulated in memory cells and targeting CD45RB with a specific antibody has been shown to inhibit graft rejection. Its role in immunity to infection, however, has not been tested. Here, we report the effect of anti-CD45RB antibody treatment on the induction of anti-influenza CD8+ T cells and viral clearance. Anti-CD45RB-treated mice had delayed pulmonary viral clearance compared with untreated mice whose infection was completely cleared by day 8 post-infection. In anti-CD45RB-treated mice, the total CD4+ and CD8+ T cell numbers in both the lungs and mediastinal nodes were substantially reduced at days 5 and 8; this effect was less marked for the spleen. CD8+ T cells specific for influenza virus were also reduced compared with the control group in all three organs at day 8. By day 11, when both treated and control groups showed no virus remaining in the lungs, specific CD8+ T cell numbers were at similar low levels. Homing to lymph nodes and lung of dye-labeled T cells was greatly inhibited (by >80%) by anti-CD45RB treatment. This reduced homing corresponded with reduced CD62L and β1-integrin expression in both uninfected and infected mice. Since CD62L plays a critical role in homing lymphocytes to lymph nodes, and high levels of CD62L and α4β1-integrin are expressed by lymphocytes that home to bronchus-associated lymphoid tissue, we suggest that reduced expression of these molecules is a key explanation for the delay in immune responses.

Introduction

Cell-mediated adaptive immune responses are critical for the recovery of primary influenza infection (1). The virus-specific CD8+ CTLs are thought to be the key effectors in the clearance of the virus especially in the first 8 days (2) and potentially provide heterologous immunity by recognizing epitopes that are highly conserved between different strains of influenza virus (3). CD4+ T-cell help is relatively dispensable in the induction of virus clearing CD8+ T cells during a primary influenza infection (4), although it may be critical for an optimal CD8+ T cell recall response during secondary infection (5–8). As we have focused on the primary infection, we have concentrated our investigations on CD8+ T cells.

CD45 is expressed on all nucleated hematopoietic cells and is a receptor tyrosine phosphatase critical for antigen-specific B and T cell responses (9, 10). Due to alternative splicing, CD45 can exist in multiple isoforms (CD45RA, RB, RC and RO) that can be differentiated by their sizes. Although the functional relevance of these isoforms is not determined, they are highly regulated and T cell activation is associated with a shift from high- to low-molecular weight isoforms. In the mouse, naive peripheral T cells express abundant CD45RB and low-level expression is seen on primed/memory T cells, on cells that show increased secretion of T helper 2 cytokines, such as IL-4 and IL-10, and on a population of T cells with regulatory function (11, 12). These assignments are useful but may be a slight over-simplification as the CD45 isoform expression is not necessarily fixed (13, 14).

Studies in transplantation indicate that treatment with the anti-CD45RB mAb, MB23G2, induces long-term tolerance to allogeneic grafts in mice (15–18).
perturbed by this antibody and the cells remaining after anti-
CD45RB treatment have little or no CD45RB. One interpretation
of these studies could be that the action of the antibody was to
deplete CD45RB+ cells. However, the possibility of altered
trafficking or sequestration of CD45RB+ cells could not be
discounted. Although anti-CD45RB treatment is not associated
with activation (CD69, CD25 and CD44 are not affected), it is
associated with increased production of both Th2 cytokines
and increased CTLA4 surface expression on CD4+ T cells (17).
This increase in CTLA4 may be critical in mediating tolerance
(17) and/or reflect a common splice pathway between CD45
and CTLA4 (19).

General immunosuppression by drugs (e.g. cyclosporin,
FK506, rapamycin and mycophenolate mofetil) has been used
clinically to inhibit transplantation rejection successfully;
however, one of the adverse effects of such drugs is increased
susceptibility to serious infections (20, 21). Anti-CD45RB
appears to be less severe than most immunosuppressive
regimens. For example, it does not deplete T cell numbers in
the spleen (17, 22). Surprisingly, the effect of anti-CD45RB
treatment on viral or bacterial immunity has not been reported.
Therefore, we wanted to investigate how such a regimen
would affect the host’s responses to adventitious infections,
e.g. influenza.

Several observations that suggest a link between CD45
expression and trafficking have been reported. Firstly, CD45
has some regulatory role in selectin expression including
CD62L (23) and in integrin-mediated adhesion (24). Secondly,
although anti-CD45RB results in an increase in splenic T cells,
there is also a decrease in circulating lymphocytes (15, 16) and
lymph node T cells (22, 25). Such imbalance in cell numbers
among the various sites could be interpreted as due to
a trafficking aberration. As specific T cells can be more readily
quantified in the murine influenza system than in allogeneic
systems, the model we have chosen also allows trafficking of
antigen-specific T cells to be examined.

In this study, the ability of mice following anti-CD45RB
treatment to induce effective CD8+ T cell-mediated viral
clearing and anti-viral antibody responses during a primary
influenza infection is reported. We also relate this to how T cell
trafficking is affected after anti-CD45RB treatment.

Methods

Mice and anti-CD45RB treatment

C57BL/6 female mice were obtained from the Department of
Microbiology and Immunology, The University of Melbourne
(Parkville, Victoria, Australia). CD4-deficient GK transgenic
mice (26) on a C57BL/6 background were obtained from the
Walter and Eliza Hall Institute (Parkville, Victoria, Australia).
The animals were bred and maintained throughout the
experiment under specific pathogen-free conditions in the
same department. The anti-CD45RB mAb, MB23G2, was
purified on immobilized protein G from hybridoma superna-
tant. Mice were injected intraperitoneally with 0.2 mg of
MB23G2, 1 day prior to infection and thereafter every 3–4
days. The control mice were injected with either isotype control
(GL117 = rat IgG2a) or PBS.

Virus infection

The type A strain of influenza virus used in this study was
a genetic reassortant of A/ Memphis/1/71 (H3N2) x A/Bellamy/
42 (H1N1) referred to as Mem71 (H3N1) virus. The split
Mem71 virus for use in ELISA was prepared by rate zonal
centrifugation to purify the virus before inactivation with
(β-propiolactone and disruption with sodium taurodeoxycho-
late. Mice were infected intranasally with 10^{4.5} plaque-forming
units of Mem71 virus after being anesthetized with penthrane.
Each mouse received 50 µl of the virus in the form of allantoic
fluid diluted in RPMI-1640.

ELISPOT (Enzyme linked immunospot) assay for
IFN-γ-secreting CD8+ T cells

Influenza-specific IFN-γ-secreting CD8+ T cells were enumer-
ated by an ELISPOT assay as previously described (27) with
2 µg ml^{-1} of a peptide representing the dominant H-2D^{b}-
restricted epitope NP_{366–374}, ASNENMETM (28).

Flow cytometric analysis of lymphocytes

Lungs were minced, digested in 2 mg ml^{-1} collagenase A
(Roche, Mannheim, Germany) for 30 min at 37°C, and then
dispersed through a metal sieve. The resultant cell suspensions
were pelleted and washed. Liver lymphocytes were
obtained as described (29). Briefly, livers were minced and
pushed through a metal sieve. Hepatocytes were removed by
centrifugation in a 36% solution of isotonic Percoll in PBS, and
the pellet containing lymphomyeloid cells and RBC was
recovered. RBCs were removed from suspensions of liver,
lung and spleen cells by lysis in Tris-buffered ammonium
chloride solution. In experiments where liver lymphocytes
were analyzed, mice were perfused by injecting 20 ml of PBS
into the heart prior to recovery of organs.

CD8+ and CD4+ T cells were enumerated by flow cytometry
using a FACScan (Becton Dickinson, San Jose, CA, USA) after
staining the lymphocytes with FITC-conjugated rat anti-mouse
CD8 antibody (clone 53-6.7, Pharmingen, San Diego, CA,
USA) and PE-conjugated rat anti-mouse CD4 antibody (clone
GK1.5, Pharmingen). Data were analyzed using FlowJo
version 3.6.1 (Tree Star Inc., San Carlos, CA, USA). Expression
of β7-integrin and L-selectin on CD8+ T cells was examined by
co-staining with PE-conjugated anti-CD8 antibody (clone
CIT-CD8a, Caltag Laboratories, Burlingame, CA, USA) and either
FITC-conjugated anti-β7-integrin antibody (Pharmin-
gen, clone M293) or FITC-conjugated anti-L-selectin antibody (clone
MEL-14, Pharmingen). Expression of β1-integrin on CD8+ T
cells was examined by co-staining with PE-Cy5-conjugated
anti-CD8 antibody (clone 53-6.7, Pharmingen) plus FITC-
conjugated anti-β1-integrin antibody (clone HA25, Pharmin-
gen). Expression of CD45RB was examined with a biotinylated
mAb that did not compete with MB23G2 (clone 16A,
Pharmingen). Propidium iodide staining was used to exclude
dead cells. Cells were analyzed using a FACScan and
CellQuest software.

ELISA

To determine the titer of influenza-specific total and isotype
(IGM, IgG1, IgG2c and IgG2b) antibodies in the sera of
influenza-infected mice, flat-bottom 96-well polystyrene 


Anti-CD45RB alters T cell homing and viral clearance

Results

Clearance of influenza virus in mice after anti-CD45RB treatment

To investigate the effect of anti-CD45RB on anti-influenza immunity that leads to viral clearance in the lungs, mice were treated intraperitoneally with 0.2 mg of anti-CD45RB 1 day prior to intranasal infection of influenza virus, and every 3 days thereafter. Control mice were either injected with PBS or the control isotype GL117; both controls behaved similarly. The ability of these mice to clear the pulmonary virus was examined 5, 8 and 11 days post-infection by plaque assay on samples from the lungs. On day 5, prior to the recruitment of CD8+ effector T cells to the lungs, viral titers were high in both groups (Fig. 1). By day 8, control mice had completely cleared the virus in their lungs (Fig. 1). In contrast, mice treated with anti-CD45RB showed a delayed viral clearance, with individual mice displaying a range of viral titers in their lungs. All the treated mice ultimately cleared the virus by day 11 post-infection. Thus, anti-CD45RB hindered but did not abrogate the ability of the mice to clear a pulmonary influenza infection.

Expansion of lymphocytes in the mice following influenza infection

During infection, the rapid proliferation of lymphocytes is important for an efficient recovery from pulmonary influenza. Here, in an attempt to explain the delayed viral clearance in mice treated with anti-CD45RB, the expansion of the lymphocytes in response to the infection was examined. The total numbers of CD4+ T cells and CD8+ T cells in the MLN, lungs and spleen of mice on days 5, 8 and 11 are shown in Fig. 2. On day 5 post-infection, in the MLN and lungs, the total numbers of lymphocytes were significantly higher for control mice than for anti-CD45RB-treated mice. This early expansion of lymphocytes in the control mice is consistent with their ability to completely clear virus within the next 3 days. As expected, on day 8 post-infection, control mice that had just cleared the virus had very high numbers of CD4+ and CD8+ lymphocytes in the lung and MLN and these levels had decreased by day 11 in the absence of further stimulus. The rise in T cell numbers on day 8 post-infection was particularly notable in the lungs.

Statistical analysis

Statistical analysis was performed using the non-parametric Mann–Whitney U test on the Prism 4 software program (GraphPad Software Inc., San Diego, CA, USA).

Fig. 1. Anti-CD45RB treatment delays virus clearance. Influenza virus load in the lungs of control and anti-CD45RB-treated mice was determined on days 5, 8 and 11 after intranasal infection. The geometric mean titer of a group is shown as a line, and each symbol represents an individual mouse. Log_{10} 1.6 is the minimum detectable titer.
where the viral clearing activity was taking place (CD4+ T cells, \( P = 0.008 \); CD8+ T cells, \( P = 0.008 \)). When the mice were treated with anti-CD45RB, fewer T cells were present in both MLN and lung. This deficit was particularly dramatic in the MLN (day 8: CD4+ T cells, \( P = 0.008 \); CD8+ T cells, \( P = 0.016 \) and day 11: CD4+ T cells, \( P = 0.016 \); CD8+ T cells, \( P = 0.008 \)). In contrast to the MLN and lung, the difference in T cell numbers in the spleens between anti-CD45RB-treated and untreated mice through most of the study was unremarkable. Only for the CD8+ T cells and only on day 8 post-infection (when there was also a maximum increase in antigen-specific CD8+ T cells; Fig. 3) was there a significant difference. The general lack of changes in total T cell numbers in the spleen is consistent with recent reports that anti-CD45RB treatment did not decrease the splenic T cell numbers (17, 22). This modest effect on spleen compared with lymph nodes was one of the early indications that there may be altered trafficking.

**Influenza-specific CD8+ T cell responses in the mice**

Early control of primary influenza infection involves CD8+ T cell-mediated immunity. Influenza-specific IFN-\( \gamma \)-secreting CD8+ T cells were enumerated in the MLN, lungs and spleen of the mice on the various days after viral infection using an ELISPOT assay (Fig. 3). The numbers of antigen-specific CD8+ T cells increased considerably between day 5 and day 8 post-infection in all organs. On day 8, the control mice had an abundance of influenza-specific IFN-\( \gamma \)-secreting CD8+ T cells in their MLN, lungs and spleen, correlating with their ability to clear the virus (Fig. 1). However, mice treated with anti-CD45RB had significantly fewer influenza-specific IFN-\( \gamma \)-secreting CD8+ T cells in the MLN (\( P = 0.008 \)), lungs (\( P = 0.032 \)) and spleen (\( P = 0.008 \)) as compared with the controls. These data parallel the reduced total CD8+ T cell numbers (Fig. 2) in the MLN and lungs of anti-CD45RB-treated mice. Nevertheless, influenza-specific IFN-\( \gamma \)-secreting CD8+ T cells were present in the virus-infected lungs of all the mice. In order to have an estimate of what proportion of CD8+ T cells was activated to secrete IFN-\( \gamma \), we divided the ELISPOT numbers in Fig. 3 by the total CD8+ T cell numbers in Fig. 2 (Table 1). The numbers are so low on day 5 that they essentially correspond to uninfected mice. There were no statistical differences in the proportion of specific T cells to total CD8+ T cells for treated
compared with control mice in the various organs on day 8. By day 11 in the MLN, this proportion was actually higher for anti-CD45RB-treated mice (11% versus 1%; \( P < 0.01 \)), even though the total numbers of influenza-specific cells in the organ were similar between treated and control mice (Fig. 3). The finding that total CD8+ T cell numbers were dramatically reduced by anti-CD45RB without a substantial decrease in the proportion of specific versus total CD8+ T cells argues against any major effect of anti-CD45RB on T cell activation, but would be consistent with a lack of recruitment.

By day 11 post-infection, the numbers of influenza-specific IFN-\( \gamma \)-secreting CD8+ T cells in the lungs in untreated mice were greatly reduced, as the immune response subsided after viral clearance (Fig. 1). In the anti-CD45RB-treated mice, there was a modest increase of anti-viral CD8+ T cells such that on day 11 there were more CD8+ T cells in the anti-CD45RB mice than the controls. This could be due in part to the delayed kinetics of the immune response in anti-CD45RB-treated mice, but could also be in part due to the infection lasting beyond day 8 in these mice. In conclusion, the observed diminished CD8+ T cell response after anti-CD45RB treatment on day 8 post-infection correlates with the delayed viral clearance in these mice. However, all anti-CD45RB-treated mice had ultimately cleared the pulmonary influenza by day 11 post-infection, despite the lack of a strong peak in CD8+ T cell number as observed in control mice on day 8 post-infection.

**Table 1.** Number of ELISPOTs per 100 CD8+ T cells MLN, lungs and spleens of mice treated with anti-CD45RB as compared with controls (\( n = 6 \))

<table>
<thead>
<tr>
<th>Post-infection</th>
<th>Treatment</th>
<th>MLN</th>
<th>Lung</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 days</td>
<td>PBS</td>
<td>0.2 ± 0.1</td>
<td>2.2 ± 2.8</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Anti-CD45RB</td>
<td>0.1 ± 0.1 (( P = 0.02 ))</td>
<td>1.2 ± 1.7 (( P = 0.55 ))</td>
<td>0.03 ± 0.02 (( P = 0.31 ))</td>
</tr>
<tr>
<td>8 days</td>
<td>PBS</td>
<td>1.1 ± 0.5</td>
<td>63.5 ± 33.1</td>
<td>1.1 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>Anti-CD45RB</td>
<td>0.6 ± 0.5 (( P = 0.10 ))</td>
<td>131.4 ± 102.7 (( P = 0.22 ))</td>
<td>0.7 ± 0.3 (( P = 0.31 ))</td>
</tr>
<tr>
<td>11 days</td>
<td>PBS</td>
<td>0.7 ± 0.4</td>
<td>38.2 ± 38.5</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Anti-CD45RB</td>
<td>11.3 ± 6.6 (( P = 0.01 ))</td>
<td>51.8 ± 27.6 (( P = 0.31 ))</td>
<td>0.3 ± 0.2 (( P = 0.84 ))</td>
</tr>
</tbody>
</table>
Taken together, these data propose that anti-CD45RB treatment delays the induction of the humoral response against influenza virus and suggest a possible role of influenza-specific antibodies in the clearance of virus observed in the anti-CD45RB-treated mice on day 11 post-infection in the absence of a strong CD8+ T cell response.

Altered T cell homing after anti-CD45RB treatment

We have shown above that the numbers of lymphocytes (especially CD8+ T cells) that gather at the site of infection are dramatically reduced. The lymphocytes that home to the bronchus-associated lymphoid tissue have high levels of CD62L and α4β7-integrin on their surface (33), whereas gut-homing lymphocytes bear high levels of α4β7-integrin (34). However, there is no such preference in the spleen, so we decided to investigate the effect of anti-CD45RB antibody treatment on the homing molecules CD62L (L-selectin), β7 and β1 on CD8+ T cells in this organ. On day 8 after infection, anti-CD45RB treatment resulted in far fewer cells bearing high levels of these homing molecules: for CD62L from 66.6% to 39.6% and for β1-integrin from 35.6% to 22.6% (Fig. 5).

In uninfected mice (n = 3), the reduction was, respectively, from 87.6% to 44.6% and from 12.6% to 9.6%. Interestingly, cells with high levels of β7-integrin are also reduced, indicating that perhaps homing to the gut might also have been altered. The effect of the anti-CD45RB treatment on homing molecules was not simply due to deletion of CD45RB cells. Firstly, there was little reduction of CD45RB+ cells in the spleen (data not shown). Even in the lymph nodes, there was a substantial proportion (31.6%) of CD45RB+ cells remaining after anti-CD45RB treatment versus the 67.8% in control mice (Fig. 6). In contrast, only 11.9% of cells remained CD62L+ (Fig. 6) and therefore most CD45RB+ cells must have down-modulated their CD62L.

To determine the effect of anti-CD45RB antibody on homing more directly, we tracked dye-labeled CD8+ T cells in vivo. CD8+ T cells from naive mice were labeled with the dye CFSE and injected intravenously. The numbers of CFSE-labeled cells that tracked to the spleen, lung, draining MLN,
non-draining ILN and liver all decreased with anti-CD45RB treatment (Fig. 7). Although the untreated donor CD8 T cells appeared homogeneous with respect to expression of CD45RB and CD62L (Fig. 7a), treatment with anti-CD45RB antibody preferentially reduced their ability to traffic to lungs and lymph node compared with spleen. Thus, in infected mice, anti-CD45RB treatment reduced trafficking to the spleen by 53% compared with a reduction in excess of 80% in lungs and lymph node (lung, 84%; MLN, 81%; ILN, 90%; Fig. 7b). Similarly, in uninfected mice, there was a 69% reduction in spleen compared with a reduction of at least 87% in lungs and lymph node (lung, 87%; MLN, 95%; ILN, 97%; Fig. 7b).

**Anti-CD45RB treatment directly reduces expression of CD62L on CD8 T cells**

The overall reduction in CD8 T cell recovery in anti-CD45RB-treated mice (Fig. 7) indicated that this antibody can deplete CD8 T cells or results in their being sequestered in an as yet unidentified site. Thus, it was important to determine if anti-CD45RB simply depletes/sequesters naive CD8+ T cells expressing high levels of CD62L/CD45RB or is also able to directly affect expression of CD62L. CD62L levels were clearly decreased upon *in vitro* incubation of purified CD8 T cells with anti-CD45RB antibody (Fig. 8). Notably, a concurrent decrease in CD45RB expression was not observed (Fig. 8).

**Discussion**

The effect of the anti-CD45RB mAb, MB23G2, on primary influenza infection was studied here. Virus-specific CD8+ T cell trafficking to lymph nodes and lung. Lymph node cells were harvested from naive syngeneic female donors and analyzed for CD62L and CD45RB expression on CD8 cells (Fig. 7a). The donor cells were then labeled with CFSE dye and adoptively transferred to mice immediately prior to either anti-CD45RB or isotype control antibody treatment. The mice were infected the following day. The spleen, lungs, MLN, ILN and liver of recipient mice were recovered on day 6 post-transfer and analyzed by flow cytometry. The number of CFSE-labeled CD8+ T cells in anti-CD45RB mice was expressed as a percentage of the number in isotype control–treated mice. The means and standard deviations of absolute numbers in isotype control–treated mice were the following—infected: spleen, 57 269 (19 324); lung, 1882 (747); MLN, 4740 (3128); ILN, 4667 (407); and liver, 472 (120); and uninfected: spleen, 65 091 (11 337); lung, 1032 (334); MLN, 90 (52); ILN, 19 096 (14 854), and liver, 743 (352).

**Fig. 7.** Anti-CD45RB treatment dramatically reduces CD8+ T cell trafficking to lymph nodes and lung. Lymph node cells were harvested from naive syngeneic female donors and analyzed for CD62L and CD45RB expression on CD8 cells (Fig. 7a). The donor cells were then labeled with CFSE dye and adoptively transferred to mice immediately prior to either anti-CD45RB or isotype control antibody treatment. The mice were infected the following day. The spleen, lungs, MLN, ILN and liver of recipient mice were recovered on day 6 post-transfer and analyzed by flow cytometry. The number of CFSE-labeled CD8+ T cells in anti-CD45RB mice was expressed as a percentage of the number in isotype control–treated mice. The means and standard deviations of absolute numbers in isotype control–treated mice were as follows—infected: spleen, 57 269 (19 324); lung, 1882 (747); MLN, 4740 (3128); ILN, 4667 (407); and liver, 472 (120); and uninfected: spleen, 65 091 (11 337); lung, 1032 (334); MLN, 90 (52); ILN, 19 096 (14 854), and liver, 743 (352).

**Fig. 8.** *In vitro* treatment with anti-CD45RB antibody results in decreased expression of CD62L on CD8 T cells. Purified CD8 T cells were incubated with 0.2 μg ml⁻¹ of either MB23G2 anti-CD45RB (open histogram) or GL117 isotype control (closed histogram) antibody for 22 h. FACS analysis was performed at the conclusion of culture. Data shown for CD8+ propidium iodide− gated lymphocytes.
T cells are known to be critical for the recovery of the early phase of influenza infection (2). In the murine influenza pneumonia model at the time of virus clearance, CD8 \(^+\) T cells dominate the cellular population in the bronchoalveolar lavage (35). Mice that were treated with anti-CD45RB demonstrated a delayed viral clearance response in which significant viral load could be detected in the lungs of these mice on day 8 post-infection, while the control mice had completely cleared the pulmonary influenza. This delayed clearance corresponded with the significant reduction in numbers of influenza-specific CD8 \(^+\) T cells in the lungs and MLN of the anti-CD45RB-treated mice.

Our findings suggest that the anti-influenza-specific CD8 \(^+\) T cell response leading to viral clearance is altered. This is concordant with studies that reported the perturbation of T cell response after anti-CD45RB treatment in allograft models (15, 16). The most marked effect of anti-CD45RB treatment in our study was the reduction in lymphocyte numbers in the MLN. In contrast, in the spleen, numbers were either unaltered or modestly reduced (Fig. 2). This resulted also in reduced numbers of influenza-specific CD8 \(^+\) T cells in the whole MLN, but as a percentage of CD8 \(^+\) T cells, there was no significant reduction (Table 1). This hinted that cell activation per se was not markedly affected by anti-CD45RB treatment and that the effect may be due to reduced naive cell trafficking to the regional lymph node for priming. For confirmation, we showed that the numbers of lymphocytes with high levels of the adhesion molecules associated with homing to lymph nodes were reduced (Fig. 5). The homing molecules we considered, L-selectin, \(\alpha_4\beta_1\) and \(\alpha_4\beta_7\), are important for homing from the blood to the high endothelial vessels of MLN (33); the latter two are also important in trafficking to the lungs (36). Less is known about what adhesion molecules are required (if any) for lymphocytes from afferent lymph; however, they comprise only 10% of lymphocytes entering lymph nodes, whereas blood lymphocytes comprise >85% (37). We contend that the reduction in cells expressing these homing molecules is not merely due to depletion/sequestration of naive cells. Firstly, there is very little depletion in the spleen. Secondly, the moderate reduction in numbers of CD45RB \(^+\) cells in lymph nodes cannot explain the dramatic reduction in numbers of CD62L \(^+\) cells (Fig. 6). Thirdly, CD62L expression was reduced under conditions where neither preferential depletion nor sequestration of CD45RB-high cells occurred viz. in vitro incubation with anti-CD45RB antibody (Fig. 8). Fourthly, the anti-CD45RB antibody used is a rat IgG2a and this isotype is inefficient at depletion (38), probably because its hinge region does not contain the Leu-Leu-Gly-Gly-Pro motif (39).

As well as the surface phenotype studies, functional studies of homing by CFSE-labeled lymphocytes (Fig. 7) showed that the greatest reduction of homing was to the lymph node after anti-CD45RB treatment. The impaired recruitment of naive cells entering the nodes thus explains the dramatic decrease in CD4 \(^+\) and CD8 \(^+\) T cell numbers in the MLN of the anti-CD45RB-treated mice (Fig. 2). This in turn would lead to reduced numbers of specific T cells being primed and hence (at least in part) delayed viral clearance. In support of our findings, a single dose of anti-L-selectin antibody treatment has been shown to delay the response to Sendai virus (40). Interestingly, mice that transgenically expressed only CD45RO did not have any alteration in lymphocyte distribution and were able to clear influenza infection by day 9 (41). We assume that some compensation has happened during lymphocyte development that allowed normal homing.

Lymphocyte trafficking as a modality for controlling immune responses has received much attention recently, especially with the uncovering of the molecular basis of the immunosuppressive drug FTY720 (42, 43). This drug blocks egress of lymphocytes from lymph nodes by its action on the sphingosine-1-phosphate 1 receptor. In contrast to egress, we have shown that anti-CD45RB antibody treatment results in reduced ingress of lymphocytes to lymph nodes. We therefore speculate that the combination of FTY720 and anti-CD45RB would have an even more dramatic effect on immune responses.

Interestingly, by day 11 post-infection, mice treated with anti-CD45RB ultimately cleared the pulmonary infection. Although the homing mechanism to the draining MLN of the lungs appears affected after anti-CD45RB treatment, the suboptimal CD8 \(^+\) T cell response that was observed in these animals could have been mostly induced in the spleen (44). This splenic population of activated CD8 \(^+\) T cells might be sufficient to generate CTL effectors needed for viral clearance eventually. This is because the optimal CD8 \(^+\) T cell response seen in control mice is probably in excess and a more modest response might be able to ultimately control the pulmonary influenza (45). The increase of anti-influenza antibody in anti-CD45RB-treated mice on day 11 post-infection could also aid in viral clearance, as the humoral response is also known to play a role in the recovery of influenza (46, 47).

The effect of anti-CD45RB treatment on the humoral response against the influenza infection was also analyzed here. Mice treated with anti-CD45RB showed a delayed humoral response on day 8 post-infection but total anti-influenza antibody titer increased dramatically by day 11 post-infection. Though CD45 is known to play a role in B cell activation by mediating signals via the B cell antigen receptor (9), the mechanism in which anti-CD45RB treatment affects the humoral response is not well studied. As B cells share many of the homing receptors of T cells (e.g. CD62L, \(\alpha_4\beta_1\)), the treatment of anti-CD45RB could also have impaired the trafficking of B cells and resulted in the delayed humoral response seen. We did not find significant differences in the isotypes of the total anti-influenza antibody between the anti-CD45RB-treated mice and the controls, even though anti-CD45RB treatment has been shown to enhance the production of Th2 cytokines (15, 25).

Interestingly, treatment of anti-CD45RB has been reported to show greater immunosuppression and enhanced graft tolerance when accompanied with the blockade of B7/CD28 and CD40/CD40L co-stimulatory pathways (22, 25). Moreover, anti-CD45RB treatment is shown to up-regulate CTLA4 expression on T cells that block B7/CD28 co-stimulation (17). To prevent allograft rejection in clinical transplantation, immunosuppression is mediated usually by a combination of drugs (e.g. FK506, mycophenolate mofetil) that is broad spectrum. As such, one of the adverse effects is increased susceptibility to infections (20, 21). Thus, one of the main aims in transplantation is to have more selective immunosuppression and anti-CD45RB may be a candidate. If anti-CD45RB were ever to be used clinically in transplant patients, it would...
be useful to know whether the host could resist infections. We have shown here that although influenza clearance was slower (by up to 3 days), the host was still able to eliminate the infection. Thus, anti-CD45RB may be a more strategic form of immunosuppression than the current drugs employed.

The treatment of anti-CD45RB has been demonstrated here to impede viral clearance of pulmonary influenza by affecting the induction of an optimal CD8+ T cell response and causing a delay in the humoral response. Although the pulmonary infection was eventually cured in the absence of an optimal induction of CD8+ T cell response, this should be seen in the light of primary influenza infection. Recent experiments have shown that optimal induction of memory CD8+ T cells requires the interplay of various immune components such as CD4+ T cells and appropriate co-stimulation (5, 6, 8, 48, 49). This is vital for vaccination schemes to provide long-term protection. Thus, it will be interesting to examine the effect of anti-CD45RB treatment on secondary viral infection, although long-term studies with the anti-CD45RB mAb would be difficult, as mice eventually make antibody against the rat mAb rendering it ineffective.

The insights on the roles of CD45RB in the induction of immune response will define important parameters in both transplantation and vaccination. In summary, this study indicates that CD45RB+ cells are critical in the optimal induction of influenza-specific CD8+ T cells response but are not mandatory for viral clearance.

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Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CFSE</td>
<td>5,6-carboxyfluorescein diacetate succinimidyl ester</td>
</tr>
<tr>
<td>ILN</td>
<td>inguinal lymph nodes</td>
</tr>
<tr>
<td>MLN</td>
<td>mediastinal lymph nodes</td>
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References


