Noncognate Interaction with MHC Class II Molecules Is Essential for Maintenance of T Cell Metabolism to Establish Optimal Memory CD4 T Cell Function

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Noncognate Interaction with MHC Class II Molecules Is Essential for Maintenance of T Cell Metabolism to Establish Optimal Memory CD4 T Cell Function

Alessandra De Riva,* Christine Bourgeois,* George Kassiotis, † and Brigitta Stockinger2*

CD4 memory T cells surviving in the absence of MHC class II contact lose their characteristic memory function. To investigate the mechanisms underlying the impaired function of memory T cells in the absence of MHC class II molecules, we analyzed gene expression profiles of resting memory T cells isolated from MHC class II-competent or -deficient hosts. The analysis focused on five transcripts related to T cell activation, metabolism, and survival that are underexpressed in resting memory T cells from MHC class II-deficient hosts compared with MHC class II-competent hosts. CD4 memory cells isolated from MHC class II-deficient hosts display alterations in their degree of differentiation as well as metabolic activity, and these changes are already manifest in the effector phase despite the presence of Ag-expressing dendritic cells. Our data suggest that the absence of interactions with noncognate MHC class II molecules compromises the progressive accumulation of signals that ensure optimal survival and fitness to sustain the metabolic activity of activated T cells and shape the functional capacity of the future memory compartment.

Materials and Methods

Mice

Female A1 TCR-transgenic Rag1–/– mouse (H2b) (21) were used between 6 and 10 wk of age. Recipient mice were either allogeneic H2Rag2+/–Il2rg+/– (referred to as MHC class II-competent mice) or allogeneic H2Rag2+/–Il2rg+/–H-2-AK–/– (referred to as MHC class II-deficient mice). CBA and C57BL/6 mice were used to provide bone marrow-derived DCs. All animals were bred under specific pathogen-free conditions, and all experiments were done in conventional but pathogen-free facilities in accordance with institutional guidelines and Home Office regulations.

Generation of effector and memory T cells

Lymph node T cells (2–4 × 106) from female A1 TCR-transgenic H2Rag1–/– mice were cotransferred with syngeneic bone marrow-derived DCs pulsed with 1 μM H-Y peptide into allotopic adoptive hosts which were either H2Rag2+/–Il2rg+/– (MHC class II competent) or H2Rag2+/–Il2rg+/–H-2-AK–/– (MHC class II deficient). DCs were generated from the bone marrow of syngeneic (CBA) or allotopic (C57BL/6) mice by culture with GM-CSF as previously described (22).

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Optimal Memory CD4 T Cell Function1


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Cell suspension, flow cytometry, and cell sorting

Lymph nodes and spleen cell suspensions were prepared in IMDM (Sigma-Aldrich). DC isolation was performed using Liberase CI Purified Enzyme Blend (Roche). All Abs were purchased from e-Bioscience with the exception of Peps from Texas Red anti-CD4 from Caltag Laboratories; biotin anti-mouse IgG (H+L), PE anti-IL-2, PE anti-DO.11.10, FITC-conjugated Armenian hamster anti-mouse Bc-2 mAb, and isotype control from BD Pharmingen; phospho-S6 ribosomal protein (Ser235/236) and phospho-Stat5 (Tyr694) primary Abs from Cell Signaling Technology; and FITC-labeled mouse H-2Kk, PE anti-IL-2, PE anti-DO.11.10, FITC-conjugated Armenian hamster anti-mouse Bc-2 mAb, and isotype control from BD Pharmingen; phospho-S6 ribosomal protein (Ser235/236) and phospho-Stat5 (Tyr694) primary Abs from Cell Signaling Technology; and FITC-labeled goat anti-rabbit IgG from BD Pharmingen as secondary Ab. For determination of intracellular proteins, cells were fixed in 100 μl of 2% paraformaldehyde in PBS for 15 min and permeabilized with 0.1% Nonidet-P40, PBS for 3 min, followed by staining with specific Abs. Analytical flow cytometry was conducted using a FACSCalibur (BD Biosciences), and the data were processed using FlowJo software (Tree Star). Cell sorting was done on a MoFlo cell sorter (Cytomation).

Analysis of gene expression

Resting memory CD4+ T cells recovered from pools of 8–10 mice per group were purified by FACS sorting on a MoFlo cell sorter to >98% purity. Total RNA was isolated (Qiagen) and assessed for quality and quantity on an Agilent Bioanalyser 2100 (Agilent Technologies) using a RNA 6000 Nano LabChip Kit (Agilent). Using a GeneChip Two-Cycle cDNA Synthesis Kit (manufactured by Invitrogen for Affymetrix), 600 ng of total RNA were amplified and hybridized on Mouse 430 A Plus chips (Affymetrix). The results were analyzed using GeneSpring version 7.0 software (Silicon Genetics), and genes differentially expressed with a cutoff of 1.5 were considered for further investigation.

Reverse transcription from total RNA was performed using a GeneAmp RNA PCR Core Kit (PerkinElmer). IL-2 and Glat1 gene expression was assessed using Assays-on-Demand Gene Expression Products (Applied Biosystems) on the ABI PRISM 7000 Sequence detection system (Applied Biosystems). Target gene expression was calculated using the comparative method for relative quantitation upon normalization to Hprt1 gene expression. For each experiment, data from each transcript were normalized to the expression levels on naive CD4 T cells. The raw data are deposited at www.ebi.ac.uk/arrayexpress/, accession number E-MEXP-890. Here we focused on five transcripts related to T cell activation and survival that are common name, and GenBank accession number of differentially expressed transcripts of the GeneSpring software. The five transcripts chosen for further analysis are shown in bold.

In vitro activation and cytokine production assays

A1 CD4 T cells were stimulated in 96-well plates with serial dilutions of plate-bound anti-CD3 (2C11) in the absence or presence of 10 μg/ml plate-bound anti-CD28. IL-2 production was assessed on day 2 in culture supernatant with an Alamar blue-based (23) CTL assay. For intracellular detection, cells were stimulated with 500 ng/ml phorbol dibutyrate (PdBu; Sigma-Aldrich), 500 ng/ml ionomycin (Sigma-Aldrich), and 10 μg/ml brefeldin (Sigma-Aldrich) for 6 h at 37°C before fixation.

Statistical analysis

p values were obtained using the Mann-Whitney two-tailed t test.

Results

Altered gene expression in memory cells from MHC class II-deficient hosts

CD4 memory T cells were generated by transferring naive T cells from A1 TCR-transgenic Rag-/- hosts specific for H-Y peptide in the context of H-2Eβ class II molecules together with Ag-pulsed syngeneic DCs into adoptive hosts that were either expressing allogeneic MHC (H-Y) or non-MHC allogeneic MHC (H-2Kk) (19). The allogeneic H-2Kk host MHC haplotype is neutral for the A1 TCR (24, 25); it cannot present cognate Ag to A1 T cells and neither positively nor negatively selects during thymic development. Therefore, Ag presentation is limited by the availability of syngeneic DCs, which disappear ~3 wk after transfer, thus ensuring the complete absence of antigenic stimulation (24). As a result, a pure population of resting memory CD4 T cells persists in these hosts. As previously described, A1 memory T cells recovered from MHC class II-deficient hosts show distinct functional defects upon reencountering their Ag, such as reduced capacity to produce IL-2, lack of providing help to B cells, and failure to reject H-Y expressing skin grafts (19).

### Table 1. Microarray data of gene expression in memory CD4 T cells recovered from MHC-competent and -deficient hosts

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Fold Change</th>
<th>Common Name</th>
<th>GenBank Reference</th>
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</thead>
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<td></td>
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<td>Stat5b</td>
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<td>1.968</td>
<td>Stat3</td>
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<td>NM_011364</td>
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<td>Mx4a4d</td>
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<td>0.27</td>
<td>Mx4a3</td>
<td>NM_133246</td>
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<tr>
<td>1449984_at</td>
<td>0.188</td>
<td>Cc12</td>
<td>NM_009140</td>
</tr>
</tbody>
</table>

Apoptosis and antiapoptosis

1416657_at | 2.354 | Akt1 | NM_009652 |
| 1422938_at | 1.877 | Bcl2 | NM_009741 |
| 1449193_at | 1.873 | Cdk5 | NM_009690 |
| 1448784_at | 1.503 | Tax10 | NM_020024 |
| 1427843_at | 0.391 | Kua | AB012278 |
| 1418901_at | 0.298 | Celpb | NM_009883 |

Metabolism

1453307_a_at | 2.019 | Anapc5 | AK003821 |
| 1417326_a_at | 2.009 | Anapc11 | NM_025389 |
| 1416886_at | 0.501 | Cdka2c | BC027026 |
| 1417457_at | 0.427 | Cks2 | NM_025415 |
| 1449519_at | 0.408 | Gadd45a | NM_007836 |
| 1428570_at | 0.406 | Ccnc | AK009615 |

Cytokines and cytokokines

1426205_at | 0.524 | Ppp1c1b | M27073 |
| 1448713_at | 0.475 | Stat4 | NM_011487 |
| 1417457_at | 0.427 | Cks2 | NM_025415 |
| 1428570_at | 0.406 | Ccnc | AK009615 |

Inflammatory responses

1449990_at | 3.271 | Il2 | AF065914 |
| 1449984_at | 0.188 | Cc12 | NM_009140 |

* Gene name, fold change of expression (MHC competent : MHC deficient), common name, and GenBank accession number of differentially expressed transcripts. The classification of genes is according to gene ontology biological processes of the GeneSpring software. The five transcripts chosen for further analysis are shown in bold.

To further investigate the mechanisms underlying the impaired function of memory T cells in the absence of MHC class II, we analyzed the gene expression profiles of resting memory T cells isolated from MHC class II-competent or -deficient hosts >70 days after immunization using the Affymetrix Mouse Genome 430 version 2.0 GeneChip. Approximately 400 genes were found differentially expressed in memory cells isolated from MHC-competent or -deficient hosts and a selection of transcripts is shown in Table I. The full set of raw data is available from www.ebi.ac.uk/arrayexpress/, accession number E-MEXP-890. Here we focused on five transcripts related to T cell activation and survival that are underexpressed in resting memory T cells from MHC class II-deficient hosts compared with MHC class II-competent hosts (Table I bold transcripts). Memory T cells from MHC class II-deficient hosts expressed reduced amounts of prestored transcripts for...
IL-2, compared with memory T cells from MHC-competent hosts. The transcripts for the two isoforms of Stat5, Stat5a and Stat5b, were down-regulated in CD4 memory T cells from MHC class II-deficient hosts. STAT5 phosphorylation after signaling through IL-2 and IL-7 receptors results in dimerization and translocation into the nucleus where it binds to target genes, such as the anti-apoptotic molecule Bcl-2 (26). The transcript for Bcl-2 was also underexpressed in A1 memory T cells from MHC class II-deficient hosts. The transcript for an important molecule involved in cell metabolism, survival, and signaling, Akt1 was underexpressed in memory T cells from MHC class II-deficient hosts, suggesting a potential impairment of survival.

FACS analysis verified that resting memory T cells recovered from MHC class II-deficient hosts expressed lower amounts of Bcl-2 than memory T cells recovered from MHC class II-competent hosts (Fig. 1A). Levels of Bcl-2 expression are correlated with expression of IL-7R (27) and indeed A1 memory T cells from MHC class II-deficient hosts display lower levels of IL-7R than those from MHC class II-competent hosts (Fig. 1B). Furthermore, the levels of phosphorylated Akt (Fig. 1C) and Stat5 (Fig. 1D) were reduced in memory T cells from MHC class II-deficient hosts. The expression levels for all markers were intermediate between naive T cells and memory T cells isolated from MHC class II-competent hosts.

Reduced metabolic activity in memory T cells from MHC class II-deficient hosts

The PI3K/AKT pathway has effects on multiple aspects of T cell survival and activation. Signal transduction through Akt can be assessed by measuring the level of phosphorylation of the small ribosomal S6 subunit (28), which is required for the assembly of the ribosomal complex for protein synthesis (29). In T cells, S6 phosphorylation is mainly due to S6K1 activation, which is induced by TCR stimulation in a PI3K/AKT-dependent manner. As a result, small and metabolically inactive T cells increase protein synthesis and acquire the size required for proliferation and optimal effector functions (30). Levels of pS6 on resting memory cells are similar whether they are isolated from MHC class II-competent or -deficient hosts (Fig. 2A and B, black histograms). Furthermore, the levels of phosphorylated Akt (Fig. 2C) and Stat5 (Fig. 2D) were reduced in memory T cells from MHC class II-deficient hosts. The expression levels for all markers were intermediate between naive T cells and memory T cells isolated from MHC class II-competent hosts (Fig. 2A and B). Akt also has a major role in glucose metabolism and regulates not only transcription of...
the main glucose transporter, Glut1 (31, 32), but also its cellular localization (33). Analysis of Glut1 mRNA expression from purified memory A1 T cells recovered from MHC class II-deficient and -competent hosts during in vitro restimulation with anti-CD3 and anti-CD28 showed that memory A1 T cells from MHC class II-deficient hosts did not reach the same levels of expression of Glut1 mRNA as memory A1 T cells from MHC class II-competent hosts (Fig. 2C). In accordance with our previously described defect in IL-2 production, memory T cells recovered from MHC class II-deficient hosts showed reduced IL-2 production assessed by intracellular staining after a short in vitro restimulation (Fig. 2D).

Thus, functional impairment of the IL-2 response as well as a reduction in metabolic activity suggests that the absence of MHC class II molecules compromises CD4 memory T cells on several levels. Although similar numbers of memory T cells can be recovered from both types of hosts, memory CD4 T cells surviving in the absence of MHC class II molecules divide more rapidly, assessed by BrdU labeling (19). This suggests that on a per cell basis the survival of memory CD4 T cells lacking MHC contact may be compromised in line with the observed alterations in metabolic activity. Indeed, propidium iodide staining of FACs-sorted memory T cells established that a higher proportion of memory T cells recovered from MHC class II-deficient hosts is in cell cycle and that there are more proapoptotic cells in this population (Fig. 2E).

Functional defects in memory A1 T cells lacking noncognate MHC class II interactions manifest themselves early in memory generation

To assess whether contact with noncognate MHC class II molecules was solely required during the maintenance of established memory cells or was needed also in the early phase of memory generation, we analyzed A1 T cells 14 days after adoptive transfer of naive A1 T cells with Ag-pulsed DCs into MHC-competent or -deficient hosts for the expression of Bcl-2 and IL-7R as well as functional activity. A time point of 42 days after transfer resembling the established memory phase was analyzed in parallel.

The number of T cells recovered from MHC class II-deficient hosts was not significantly different from those in MHC class II-competent hosts (data not shown). Already at day 14 after transfer/activation, A1 T cells transferred into MHC class II-deficient hosts showed intermediate expression levels of Bcl-2 between naive A1 T cells and memory A1 T cells generated in MHC class II-competent hosts (Fig. 3A). IL-7R expression (Fig. 3B) was consistently lower in A1 T cells isolated from MHC class II-deficient hosts than in A1 T cells from MHC class II-competent hosts and like Bcl-2 slightly higher than in naive T cells. Functionally, as early as day 14 after activation/transfer, A1 T cells isolated from MHC class II-deficient hosts were less efficient than A1 T cells from MHC class II-competent hosts in IL-2 production, as assessed by in vitro reactivation with anti-CD3 with or without anti-CD28 (Fig. 3, C and D). In addition, lower levels of phosphorylated S6 seen in established memory T cells also were already evident in A1 T cells isolated 14 days after transfer/activation in MHC class II-deficient hosts (Fig. 3E).

These results suggest that differences in the functional and metabolic activity of A1 memory T cells in MHC class II-deficient hosts are already imprinted in the early stages of memory generation.

FIGURE 3. Kinetics of T cell defect in memory A1 T cells generated in the absence of MHC class II. FACS analysis of Bcl-2 (A) and IL-7Rα (B) ex vivo expression of memory A1 T cells from MHC class II-competent hosts (open histograms) and MHC class II-deficient hosts (gray histograms) recovered 14 or 42 days (d) after adoptive transfer/activation. Naive A1 T cells (stippled histograms) and isotype control (black histograms) are shown as reference. Mean fluorescence intensities (MFI), SD, and p values are shown for all markers. C and D, IL-2 production by memory A1 T cells recovered 14 days after adoptive transfer/activation into MHC class II-competent hosts (open dots) or MHC class II-deficient hosts (gray squares). IL-2 production after was assessed after 2 days of in vitro stimulation with either plate-bound anti-CD3 (C) or anti-CD3 and anti-CD28 (D). Mean values and SD for four mice per group are shown. Where SDs are not visible the bars are smaller than the symbol used. E, Intracellular staining for pS6 after 30 min in vitro stimulation with PdBU and ionomycin showing memory cells from MHC class II-competent hosts (open histogram), MHC class II-deficient hosts (gray histogram), as well as resting unstimulated memory T cells (black histogram).
Altered expression of activation markers and reduced functional activity in the absence of noncognate MHC class II molecules. A1 T cells were isolated from MHC class II-competent (open histograms) or -deficient (gray histograms) hosts and analyzed at day 4 (left panels), 8 (middle panels), and 14 (right panels) for expression of CD44 (A), CD71 (B), CD98 (C), and intracellular staining for pStat5 (D). Intracellular expression of IL-2 after a 4-h restimulation with PdBu-ionomycin and brefeldin A (E). Naive A1 T cells are shown as stippled histograms and isotype controls as black histograms. Histograms represent values of cells from four mice per group.

**FIGURE 4.** Impairment in A1 T cells activation in the absence of noncognate MHC class II molecules. A1 T cells were isolated from MHC class II-competent (open histograms) or -deficient (gray histograms) hosts and analyzed at day 4 (left panels), 8 (middle panels), and 14 (right panels) for expression of CD44 (A), CD71 (B), CD98 (C), and intracellular staining for pStat5 (D). Intracellular expression of IL-2 after a 4-h restimulation with PdBu-ionomycin and brefeldin A (E). Naive A1 T cells are shown as stippled histograms and isotype controls as black histograms. Histograms represent values of cells from four mice per group.

Evidence of functional impairment as early as 14 days after transfer/activation into MHC class II-deficient hosts suggests a role for noncognate MHC contact during the effector phase of the immune response. We therefore studied the expression of activation markers 4, 8, and 14 days after transfer of naive A1 T cells with Ag-pulsed DCs into MHC class II-deficient or -competent hosts.

Similar numbers of A1 T cells were recovered from both hosts at day 4, but at day 8 A1 T cells seemed to have expanded to a lesser extent in MHC class II-deficient hosts than in MHC class II-competent hosts. On day 14 after transfer/activation, the numbers of A1 T cells recovered reflected the numbers usually recovered in the memory phase, and there was no significant difference in recovery of T cells from the two host types (data not shown). We next determined the status of activation achieved by transferred T cells by measuring the expression of CD44, IL7R-α, CD71, CD98 and pStat5 at each time point (Fig. 4). In both hosts, A1 T cells up-regulated the expression of CD44 throughout the effector phase, although A1 T cells from MHC class II-deficient hosts followed a slower kinetics than those from MHC class II-competent hosts. There was no difference in the expression of the early activation markers CD69 and CD25 at any of the time points tested (data not shown). IL7Rα expression was down-regulated to a similar degree in A1 T cells from both hosts during the acute effector stage in accordance with reduced expression of this marker after activation. CD71, the transferrin receptor, and CD98 (4F2 Ag or Ly-10), the common H chain subunit component of amino acid transporters, (34) were expressed at lower levels in A1 T cells from MHC class II-deficient hosts. This indicates a reduction in metabolic activity, which is necessary to sustain activation (and possibly proliferation). However, CD71 and CD98 expression were transient because the expression of both molecules was down-regulated in A1 T cells from both types of hosts at days 8 and 14. Stat5 phosphorylation on day 4 after transfer/activation was evident only in a subset of A1 T cells from MHC class II-deficient hosts. This indicates a reduction in metabolic activity, which is necessary to sustain activation (and possibly proliferation). However, CD71 and CD98 expression were transient because the expression of both molecules was down-regulated in A1 T cells from both types of hosts at days 8 and 14. Stat5 phosphorylation on day 4 after transfer/activation was evident only in a subset of A1 T cells from MHC class II-deficient hosts, whereas virtually all A1 T cells transferred into MHC class II-competent hosts displayed Stat5 phosphorylation at this stage.

We also tested A1 T cells isolated from MHC class II-deficient or -competent hosts for their capacity to produce IL-2 upon a short restimulation in vitro (Fig. 4F). Effector cells recovered 4 days after transfer/activation were refractory to restimulation, and no intracellular IL-2 was detected. By days 8 and 14 after transfer, A1
T cells from MHC class II-competent hosts showed a high proportion of IL-2 producers comparable with that seen in established memory A1 T cells. In contrast, A1 T cells from MHC class II-deficient hosts showed impaired IL-2 production on day 8 after activation. As expected, the reduced capability to synthesize IL-2 detected by intracellular staining following a 4-h restimulation with PdBU-ionomycin and brefeldin A. Open bar and striped bar on the left represent A1 T cells isolated from MHC class II-competent hosts without (open) or with (striped) B cells; gray bar and striped bar on the right represent A1 T cells isolated from MHC class II-deficient hosts without (gray) or with (striped) B cells. Values are the means and SDs from four mice per group. p values were obtained by Mann-Whitney U test.

**FIGURE 5.** Restoration of A1 T cell function by cotransfer of allogeneic B cells. A, Histograms show the level of phosphorylated Stat5 in A1 memory T cells from MHC class II-competent hosts (open histograms) or MHC class II-deficient hosts (gray histograms). Left, pStat5 levels in memory cells from hosts that did not receive any B cells; right, pStat5 in memory cells isolated from hosts that received cotransfer of allogeneic B cells expressing noncognate MHC class II molecules. Histograms represent values of cells from four mice per group. B, Percentage of A1 T cells expressing IL-2 detected by intracellular staining following a 4-h restimulation with PdBU-ionomycin and brefeldin A. Open bar and striped bar on the left represent A1 T cells isolated from MHC class II-competent hosts without (open) or with (striped) B cells; gray bar and striped bar on the right represent A1 T cells isolated from MHC class II-deficient hosts without (gray) or with (striped) B cells. p values were obtained by Mann-Whitney U test.

Discussion

Self-peptide recognition by mature T cells in the periphery is an important phenomenon that affects many aspects of T cell behavior, such as survival (35), homeostatic expansion, and antigen reactivity (19, 35, 36). It was demonstrated that naive CD4 T cells require continuous MHC class II contact to maintain their responsiveness to subsequent Ag stimulation. Even a short loss of this contact (as short as 20 min) resulted in a reduction in the ability to respond to Ag as measured by proliferation, IL-2 production, and determination of cell size (18, 36).

Although MHC contact seemed to be of less importance for memory T cells as far as survival and overall functionality are concerned, we had previously shown that more physiological tests of memory function based on either in vivo readouts such as skin graft rejection or on more subtle in vitro analysis clearly indicate a substantial loss of functional ability in memory cells that do not have continuous exposure to MHC class II molecules (19). These studies relied on an adoptive transfer of naive transgenic T cells together with Ag-pulsed syngeneic DCs into allogeneic hosts that were either MHC competent or MHC class II deficient. Formally, the H-2A β knockout is not completely devoid of MHC class II molecules given that these mice can form heterodimers between H-2A α and H-2B β molecules which can interact with some CD4 T cells (37). However, the presence of this hybrid form was not sufficient to ensure survival of naive A1 Tg CD4 T cells (data not shown) and to preserve survival and functionality of the A1 memory cells. Using an allogeneic adoptive transfer system allowed us to control the quality and quantity of specific Ag/MHC class II presentation, because Ag presentation was restricted to the donor bone marrow-derived DCs injected without any cross-presentation by allogeneic host DC whether or not they were expressing MHC class II molecules. The absence of the cytokine γ and Rag in these hosts furthermore guaranteed that there would be no NK-mediated rejection of the injected allogeneic T cells and DC. A criticism that is sometimes applied to adoptive transfer models into lymphopenic hosts concerns the potential for lymphopenia-driven expansion of the transferred cells due to unlimited IL-7 in the lymphopenic host. However, we have previously shown that lymphopenia-driven expansion of A1 T cells is minor in comparison with Ag-driven expansion which occurs in cotransfers of T cells and Ag-pulsed DCs (38), so that it seems unlikely that lymphopenia constitutes a major disturbance in this experimental system.

To define the basis for the functional impairment of memory T cells that lost MHC contact, gene expression profiles of resting
memory CD4 T cells generated in MHC class II-deficient or -competent hosts were compared. Among the transcripts differentially expressed, our attention was drawn to a group of genes that suggested a reduced survival capacity (Bel2 and Akt1) and a decreased potential to efficiently respond to Ag restimulation (Stat5 and II-2) in resting memory CD4 T cells recovered from MHC class II-deficient hosts. Although similar cell recovery from both types of hosts superficially suggested similar survival capacity, we had initially observed that CD4 memory T cells maintained in MHC class II-deficient hosts have higher rates of division as measured by BrdU incorporation (19). Here we show by propidium iodide staining of freshly isolated and sorted cells that memory A1 cells from MHC class II-deficient hosts not only proliferate at a higher rate than memory cells from MHC class II-competent hosts but also exhibit a higher proportion cells undergoing apoptosis. Restoration of a functional memory population was achieved by cotransfer of allogeneic MHC-expressing B cells during initiation of the T cell response. Although B cells were clearly sufficient to provide the necessary signals, it is quite likely that other MHC class II-expressing cells, notably DC, are involved in this process. Reconstitution of MHC class II expression after initiation of the effector phase (14 days after T cell transfer) did not restore a functional memory response (data not shown).

Thus, the data shown here suggest that the functional defects apparent in the memory phase are already imprinted during the effector phase despite the fact that syngeneic DCs presenting cognate Ag to the transferred T cells are maintained throughout the effector phase. The absence of allogeneic noncognate MHC class II contact in this early phase of activation weakened the metabolic activity of effector cells, impaired their survival capacity, and probably as a consequence of these events compromised their functional capacity, an effect that was imprinted in the persisting memory population. Because T cells do not all receive identical signals from APCs, heterogeneity of the effector pool is expected, and indeed it was shown that T cells accumulate signals and acquire functional fitness progressively (39). Although only agonist peptide-MHC class II complexes can initiate T cell activation, noncognate MHC class II complexes expressing self peptides also accumulate in the immunological synapse, suggesting such noncognate ligands contribute to synapse formation and T cell signaling (40). The involvement of neutral, nonselecting MHC molecules in interaction with memory T cells is not an unusual finding and has been reported previously (19, 41, 42). However, our data indicate that the requirement for noncognate MHC interaction for shaping the functional capacity of memory cells is already imprinted in the effector phase. Whether recognition of allogeneic MHC molecules involves the TCR itself or whether interaction of nonpolymorphic regions with CD4 suffices as signal is currently not clear.

Our data suggest that the absence of interactions with noncognate MHC class II molecules compromises the progressive accumulation of signals that ensure optimal survival and fitness and that AKT-mediated coordination of survival and metabolic pathways may be one of the crucial events linking signals via MHC class II molecules to the successful generation of a long-lived functional memory CD4 T cell population.

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Disclosures

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References


