Antigen-Experience CD4 T Cells Display a Reduced Capacity for Clonal Expansion In Vivo That Is Imposed by Factors Present in the Immune Host

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Antigen-Experience CD4 T Cells Display a Reduced Capacity for Clonal Expansion In Vivo That Is Imposed by Factors Present in the Immune Host

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It is thought that protective immunity is mediated in part by Ag-experienced T cells that respond more quickly and vigorously than naive T cells. Using adoptive transfer of OVA-specific CD4 T cells from TCR transgenic mice as a model system, we show that Ag-experienced CD4 T cells accumulate in lymph nodes more rapidly than naive T cells after in vivo challenge with Ag. However, the magnitude of clonal expansion by Ag-experienced T cells was much less than that of naive T cells, particularly at early times after primary immunization. Ag-experienced CD4 T cells quickly reverted to the slower but more robust clonal expansion behavior of naive T cells after transfer into a naive environment. Conversely, the capacity for rapid clonal expansion was acquired by naive CD4 T cells after transfer into passively immunized recipients. These results indicate that rapid in vivo response by Ag-experienced T cells is facilitated by Ag-specific Abs, whereas the limited capacity for clonal expansion is imposed by some other factor in the immune environment, perhaps residual Ag. The Journal of Immunology, 2000, 164: 4551–4557.

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Adoptive transfer and immunization

The behavior of naive T cells was studied by adoptive transfer (via i.v. injection) of lymph node and spleen cells from naive wild-type or SCID DO11.10 donors, containing 2.5 × 10^6 CD4^+ KJ1-26^+ cells, into unirradiated normal BALB/c mice (10). In cases where B cell-deficient mice were used as recipients, lymph node and spleen cells from the DO11.10 donors were depleted of B cells by incubating with an anti-B220 mAb (RA3-3A1/6.1, American Type Culture Collection, Manassas, VA) and rabbit complement (Accurate Chemical and Scientific, Westbury, CT). The elimination of B cells from the cell preparation was confirmed before transfer by staining with FITC-labeled goat anti-mouse Ig (Caltag, South San Francisco, CA). Recipients were immunized 1 to several days after transfer by injection of 100 μg of chicken OVA (Sigma, St. Louis, MO) or 300 μg of OVA peptide 323–339 emulsified in CFA or IFA into two s.c. sites on the back or by i.v. injection of 300 μg of OVA peptide 323–339 plus 150 μg of LPS (serotype Escherichia coli 026:B6; Difco, Detroit, MI). In some experiments bromodeoxyuridine was offered to mice in the drinking water (0.8 mg/ml) ad libitum, beginning on the day of immunization. In other experiments recipients of naive DO11.10 T cells were passively immunized by i.p. injection every day for 4 days with 1.2 mg of purified IgG from OVA/CFA-primed or naive mice, ending on the day before immunization with OVA/IFA. The titers of anti-OVA IgG1 and IgG2a Abs in the passively immunized animals were similar to those of DO11.10 recipients immunized 3 wk previously with OVA/CFA. In cases where B cell-deficient mice were used as recipients, lymph node and spleen cells from the DO11.10 strain were transferred into normal BALB/c mice (10) for the initial experiments because they contain only naive CD4 T cells that express the DO11.10 TCR.
the draining lymph nodes then fell dramatically by day 7 and slowly thereafter to a level on day 25 that was about twice the starting level. The majority of DO11.10 T cells that remained in the lymph nodes 21–25 days after immunization were no longer blasts (Fig. 1G), showed evidence of at least four cell divisions (Fig. 1F), and expressed the lower levels of CD45RB (Fig. 1C) characteristic of Ag-experienced T cells. Identical results were obtained when normal DO11.10 mice were used as donors (data not shown). Thus, the small population of pre-existing Ag-experienced cells present in normal DO11.10 donors had no detectable effect on the clonal expansion and contraction of naive DO11.10 T cells or on the production of OVA-experienced DO11.10 T cells during the primary response to OVA. For this reason, normal DO11.10 donors were used for subsequent experiments. Similar results were obtained when OVA/LPS or OVA/IFA were used as the s.c. immunogens instead of OVA/CFA (10, 19).

Clonal expansion by Ag-experienced T cells

The clonal expansion of Ag-experienced DO11.10 T cells during a secondary response was tested by challenging recipient mice with OVA at various times after primary immunization. In the experiment shown in Fig. 2A, recipients were immunized with OVA peptide/CFA, and as expected, the initially naive DO11.10 T cells increased 30-fold in the draining lymph nodes on day 5 and then fell to a lower level on day 12 that was still significantly greater than the starting level. When these recipients were injected

FIGURE 1. Ag-experienced T cells have divided in the past and changed their surface phenotype. A contour plot of expression of CD4 and the DO11.10 TCR (recognized by the KJ1-26 mAb) on lymph node T cells from a BALB/c recipient of DO11.10 T cells is shown in A. CD4⁺, KJ1-26⁺ cells, which appeared in the upper right quadrant of A, were not detected among the lymph node cells of BALB/c mice that did not receive DO11.10 T cells (10, 19). The levels of CD45RB on polyclonal BALB/c (thin line) or DO11.10 CD4 T cells (thick line) from mice that were not immunized (B) or were immunized with OVA/CFA 21 days earlier (C) are shown. CD45RB was detected with different fluorochromes that gave differing degrees of fluorescent signal in B (FITC) and C (allophycocyanin). This accounts for the difference in the shape of the histograms for polyclonal BALB/c CD4 cells, which served as internal markers for the high and low levels of CD45RB expression. Despite the difference in intensity between the fluorochromes used to detect CD45RB, it is apparent that the naive DO11.10 cells overlaid the CD45RBʰ⁺ BALB/c cells in B, and the Ag-experienced DO11.10 cells overlaid the CD45RB⁻⁺ BALB/c cells in C. D, CFSE levels in DO11.10 T cells from the draining lymph nodes of unimmunized recipients (thin line) or mice that were injected 3 days earlier with OVA/CFA (thick line). The mean number of DO11.10 T cells in the draining lymph nodes, the percentage of these cells that divided more than three times based on CFSE dilution, and the sizes of these cells at the indicated times after injection of OVA/CFA are shown in E–G, respectively. The error bars indicate the range of duplicate measurements.

FIGURE 2. Clonal expansion by Ag-specific CD4 T cells during the primary and secondary responses. A, Recipients of naive DO11.10 T cells were injected with 300 μg of OVA peptide 323–339 in CFA. Twelve days later, these mice were injected again with 100 μg of OVA peptide in IFA (indicated by the arrow). The mean number ± range of DO11.10 T cells present in the draining lymph nodes at the indicated times (n = 2 for each group and at each time point) were measured by flow cytometry. Similar results were obtained in five independent experiments. B, Naive recipients of DO11.10 T cells or DO11.10 recipients that were injected with OVA/CFA 2 wk previously were injected s.c. with OVA/IFA. Recipients were offered bromodeoxyuridine beginning on the day of OVA/IFA challenge. The mean percentage ± SEM of DO11.10 T cells that incorporated bromodeoxyuridine into DNA 5 days after OVA/IFA challenge are shown. Less than 5% of the DO11.10 T cells incorporated bromodeoxyuridine in naive or OVA/CFA-primed mice that were not challenged with OVA/IFA (17) (data not shown).
with OVA again on day 12, the now Ag-experienced DO11.10 T cells increased in the draining lymph nodes 3 days later to a maximal level that was only 3-fold higher than the number present on the day of secondary challenge. Large numbers of DO11.10 T cells were not present in the thoracic duct lymph (assessed by cannulation of the thoracic duct as described in Ref. 20), at the s.c. challenge site, or in the spleen at any time after secondary challenge (R. Merica, unpublished observation), indicating that rapid migration out of the lymph nodes was not responsible for the poor accumulation of Ag-experienced DO11.10 T cells. On the contrary, poor proliferation was involved because DO11.10 T cells in mice that received primary immunization 2 wk earlier, incorporated less bromodeoxyuridine into DNA after secondary challenge than naive DO11.10 T cells did after primary exposure to OVA (Fig. 2B). Therefore, although the Ag-experienced DO11.10 T cells accumulated maximally in the lymph nodes more rapidly after Ag challenge than naive DO11.10 T cells, the magnitude of their proliferation was much smaller.

The results of a second experiment of this type are shown in Fig. 3. The fold increase in DO11.10 T cells present in the draining lymph nodes after OVA injection is shown to correct for the different numbers of DO11.10 T cells present at the time of primary (for naive cells) or secondary (for Ag-experienced cells) immunization. Again, naive DO11.10 T cells underwent a 20-fold expansion in the draining lymph nodes that peaked 5 days after Ag injection. In contrast, DO11.10 T cells that experienced primary immunization 2 wk before secondary challenge increased only 5-fold, with maximal accumulation occurring on day 3. Ag-experienced DO11.10 cells accumulated to a greater extent after secondary challenge if 10 wk passed between the time of the first and second injections of OVA, although again the peak of clonal expansion was lower than that achieved by naive cells and occurred on day 3 instead of day 5 (Fig. 3A). Notably, DO11.10 T cells that experienced Ag 2 wk earlier possessed the memory/activated phenotype (CD45RBlow, LFA-1 high), whereas the DO11.10 T cells that experienced Ag 10 wk earlier could not be distinguished from naive T cells based on surface phenotype (Fig. 3B). Therefore, the improvement in clonal expansion potential by Ag-experienced DO11.10 T cells between 2 and 10 wk correlated with reversion to a naive cell surface phenotype.

Despite exhibiting a small degree of clonal expansion, DO11.10 T cells that were exposed to Ag 2 wk earlier were very sensitive to restimulation by Ag in vivo, requiring less Ag to achieve a maximal response on day 3 than naive DO11.10 T cells required to

**FIGURE 3.** Clonal expansion by Ag-experienced T cells at early and late times after primary immunization. A, Recipients of naive DO11.10 T cells were injected with 100 μg of OVA in CFA. Two weeks (circles) or 10 wk (diamonds) later, these mice or a naive control group (squares) were injected with 100 μg of OVA in IFA. The number of DO11.10 T cells present in the draining lymph nodes was measured by flow cytometry at the indicated times after OVA/IFA injection. These values were divided by the number of DO11.10 T cells present in each group on the day of OVA/IFA injection to calculate the fold expansion. The mean fold expansion ± SEM are shown (n = 3–4 for each group and at each time point). B, The percentage of CD4+, KJ1-26+ cells expressing the naive phenotype (high levels of CD45RB [■] or low levels of LFA-1 [□]) in each group at the time of OVA/IFA injection.

**FIGURE 4.** Ag sensitivity and IL-2 production kinetics of Ag-experienced CD4 T cells. A, Recipients of naive DO11.10 T cells were injected with 100 μg of OVA/CFA (○) or CFA alone (□). Two weeks later, the mice were injected with the indicated amounts of OVA in IFA. The percent maximal response was calculated by dividing the number of DO11.10 T cells present in the draining lymph nodes at a given Ag dose at the time of maximal clonal expansion (day 5 for the naive group [□], day 3 for the Ag-experienced group [○]) by the number present when 1 mg of OVA was injected into the naive group, and multiplying by 100. B, Recipients of naive DO11.10 T cells were not immunized (□) or were injected with OVA/LPS (○) to produce Ag-experienced T cells. Eighteen days after adoptive transfer and 17 days after OVA/LPS injection, recipients were challenged with an i.v. injection of 100 μg of OVA peptide. At the indicated times after challenge, the percentage of CD4+, KJ1-26+, IL-2-producing DO11.10 T cells was assessed by flow cytometry. The mean ± range (n = 2 at each time point) are shown.
achieve a maximal response on day 5 (Fig. 4A). In addition, Ag-experienced DO11.10 T cells produced IL-2 more rapidly in response to in vivo Ag challenge than did naive DO11.10 T cells. As shown in Fig. 4B, naive DO11.10 T cells produced IL-2 in the lymph nodes within 3 h of i.v. injection of OVA peptide, with maximal production occurring at 6 h. Approximately 25% of the DO11.10 T cells contained intracellular IL-2 at the peak of production. Naive DO11.10 T cells stopped producing IL-2 by 12 h after OVA peptide injection. In contrast, DO11.10 T cells in mice immunized 17 days earlier achieved peak production of IL-2 by 1.5 h after i.v. challenge with OVA peptide, and production declined rapidly thereafter. Therefore, although Ag-experienced DO11.10 T cells displayed a reduced capacity for clonal expansion, these cells were very sensitive to Ag stimulation and were rapid lymphokine producers in vivo.

Clonal expansion potential by Ag-experienced T cells in a naive environment

It was possible that a factor(s) present in the immune environment was responsible for the reduced clonal expansion potential of Ag-experienced DO11.10 T cells at early times after primary immunization. If this supposition was correct, then Ag-experienced T cells would have been expected to recover the capacity for robust clonal expansion after transfer into naive recipients. This was tested using a serial adoptive transfer method. Naive DO11.10 T cells were transferred into naive recipients, which were immunized with OVA/CFA. Several weeks later, purified CD4 T cells (containing $10^6$ Ag-experienced DO11.10 T cells) from these mice were transferred into a new set of naive recipients that had never been exposed to OVA. One week after cell transfer, the recipient mice were challenged with OVA. As shown in Fig. 5A, the Ag-experienced DO11.10 cells that resided in a naive environment for 1 wk displayed the robust clonal expansion pattern of naive DO11.10 T cells. The recovery of clonal expansion potential in a naive environment was accompanied by reversion of the Ag-experienced DO11.10 cells to a naive cell surface phenotype (Fig. 5B). However, the Ag-experienced DO11.10 cells that resided for 1 wk in naive hosts did not behave like naive T cells with respect to IL-2 production. Like Ag-experienced DO11.10 T cells that remained in immune hosts, the Ag-experienced DO11.10 T cells that resided in naive hosts produced IL-2 more quickly than naive cells during the first 1.5 h after in vivo challenge with OVA peptide (Fig. 5C). In addition, a larger fraction of the Ag-experienced DO11.10 T cells that resided in naive hosts produced IL-2 than did Ag-experienced DO11.10 T cells that remained in the immune environment. Therefore, Ag-experienced CD4 T cells that resided in a naive environment for 1 wk reverted to the surface phenotype and robust clonal expansion behavior of naive T cells, but retained the capacity to produce IL-2 rapidly.

It was possible that the robust clonal expansion behavior of Ag-experienced T cells that were parked in naive hosts was not explained by reversion, but, rather, by the fact that truly naive T cells that did not experience Ag during primary immunization survived preferentially in the naive hosts. The observation that Ag-experienced DO11.10 T cells that resided in naive hosts retained a rapid IL-2 potential suggested that this was not the case. Furthermore, the Ag-experienced DO11.10 T cells that resided in naive hosts for 12 days showed evidence of approximately four cell divisions (Fig. 6C) like Ag-experienced cells that remained in immune hosts (Fig. 6B), not less than one cell division like naive cells (Fig. 6A). Thus, although the DO11.10 T cells from immunized mice that were parked in naive hosts possessed a naive surface phenotype and clonal expansion potential, these cells clearly experienced Ag in the past based on their cell division history.

![FIGURE 5. Ag-experienced T cells revert to the clonal expansion behavior of naive T cells after transfer into a naive environment. Three types of adoptive transfer mice were prepared: a naive group in which naive DO11.10 T cells were transferred into naive recipients ( ), an Ag-experienced group consisting of DO11.10 T cell recipients that had been injected with OVA/CFA 3 wk previously ( ), and an Ag-experienced transfer group in which draining lymph node cells (containing $1.25-2.5 \times 10^6$ DO11.10 Ag-experienced T cells) from DO11.10 recipients that had been injected with OVA/CFA 2 wk previously were parked in naive recipients for 1 wk ( ). A, The mean fold expansion values over a prechallenge baseline $\pm$ SEM ($n = 3-16$ for each group and at each time point) of DO11.10 T cells from each group, present in the draining lymph nodes at the indicated times after s.c. challenge with OVA/CFA. B, The percentages of DO11.10 T cells expressing high levels of CD45RB ( ) or low levels of LFA-1 ( ) in the three groups on the day of rechallenge. C, The mean percentage of IL-2$^+$, CD4$^+$, KJ1-26$^+$ cells $\pm$ range ($n = 2$ for each group and at each time point) from each group, present in the spleen or lymph nodes at the indicated times after i.v. challenge with 100 $\mu$g of OVA peptide.](http://www.jimmunol.org/)

![FIGURE 6. Cell division history of Ag-experienced CD4 T cells after transfer into naive hosts. CFSE histograms are shown for the following groups: naive DO11.10 T cells that were labeled with CFSE, transferred into naive recipients, and analyzed 23 days later (A); naive DO11.10 T cells that were labeled with CFSE, transferred into naive recipients that were then injected with OVA/CFA, and analyzed 23 days later (B), and naive DO11.10 T cells that were labeled with CFSE, transferred into naive recipients that were then injected with OVA/CFA, transferred from these mice into naive recipients 11 days later, and analyzed 12 days after that (C). The mean number of cell divisions $\pm$ range ($n = 2$) are shown in each histogram.](http://www.jimmunol.org/)
Role of Ag-specific Ab in the clonal expansion of T cells

Ag-specific Ab could have been the factor in immune hosts that limited the clonal expansion potential of Ag-experienced T cells by rapidly clearing the OVA after challenge injection (21). However, DO11.10 T cells that were immunized with OVA in normal or B cell-deficient hosts underwent limited clonal expansion compared with naïve DO11.10 cells, 3 (data not shown) or 5 days after secondary challenge with OVA (Fig. 7A). Thus, Ag-specific Abs cannot account for the factor in immune mice that limits the clonal expansion potential of Ag-experienced T cells. This conclusion was supported by passive immunization experiments. Naïve DO11.10 T cells were transferred into recipient mice that had been injected with anti-OVA Abs and were then immunized with OVA/CFA. As shown in Fig. 7B, the clonal expansion of naïve DO11.10 T cells was not inhibited in recipients that had been passively immunized with anti-OVA Abs. On the contrary, the OVA-specific Abs increased the tempo of the expansion of the DO11.10 T cells, such that a maximal level was reached on day 3 instead of day 5.

Discussion

We noted that the clonal expansion potential of Ag-experienced CD4 T cells in the lymph nodes in response to secondary challenge with Ag was lower than that observed for naïve T cells during the primary response. This phenomenon is also apparent in the work of McHeyzer-Williams and colleagues (22, 23), who showed that moth cytochrome-specific naïve T cells in normal mice expand 300-fold during the primary response, whereas Ag-experienced T cells of the same specificity expanded 30-fold in response to secondary challenge. Similarly, Kerksiek et al. (24) and Kedl et al. (25) showed that certain Ag-specific CD8 T cells undergo extensive clonal expansion after primary, but not secondary, stimulation. On the other hand, Busch et al. (26) reported that the number of peptide-class I MHC-specific CD8 T cells present at the peak of the secondary response exceeded the number present at the peak of the primary response (26). However, because it was not possible to assess the frequency of naïve Ag-specific T cells present in the normal repertoire before primary immunization, it is still possible that the fold expansion of naïve T cells in the primary response exceeded that of Ag-experienced T cells in the secondary response. It is also noteworthy that the Ag-experienced CD8 T cells had reverted to high level expression of CD62L by the time the secondary challenge was performed (26). Therefore, it is possible that the Ag-experienced T cells had reverted to the more robust clonal expansion behavior of naïve T cells.

The increased potential for clonal expansion by Ag-experienced CD4 T cells long after primary immunization or after transfer into naïve recipients coincided with reversion to a naïve cell surface phenotype. Reversion of Ag-experienced CD4 T cells to a naïve surface phenotype has been observed in other studies (28–30). Garcia et al. (31) recently reported that Ag-experienced CD4 T cells retained the capacity to produce IFN-γ long after APC capable of presenting residual Ag disappeared. This result together with our findings that Ag-experienced DO11.10 T cells retained the capacity for rapid IL-2 production after transfer into the naive environment demonstrates that not all functions of Ag-experienced CD4 T cells revert to the naive state in the naive environment.

The Ag-experienced CD4 T cells that regained the capacity for robust clonal expansion 10 wk after primary immunization still

![Image of a graph showing clonal expansion by Ag-experienced CD4 T cells](http://www.jimmunol.org/Downloadedfrom)
displayed a peak response on day 3 instead of day 5 like truly naive T cells. This response pattern was similar to that displayed by naive T cells that were exposed to Ag in passively immunized recipients. Thus, 10 wk after immunization, Ag-experienced T cells may be inherently capable of the slower clonal expansion rate of naive T cells, but may respond more quickly than naive T cells because Ag-specific Abs bind the challenge Ag and efficiently direct it to APC. Ab-mediated facilitation of Ag presentation may also contribute to the capacity of Ag-experienced cells to respond to lower Ag doses than naive T cells. Ag-Ab complexes have been reported to enhance Ag presentation by binding to Fc or complement receptors (21, 32–34) on APC.

Our results are consistent with the following model of Ag-experienced T cell function. Naive CD4 T cells are stimulated by peptide-MHC-bearing APC in the lymphoid tissue to proliferate and help B cells produce Abs. Many of the activated T cells then die, leaving behind a population of Ag-experienced T cells. These cells are chronically or periodically stimulated by APC bearing small numbers of peptide-MHC molecules derived from the immunogen. This chronic signaling causes the Ag-experienced T cells to express the memory/activated phenotype and exist in a state characterized by rapid lymphokine production potential but poor proliferative capacity. If the host is challenged with Ag during this period, the Ag is rapidly bound by Ab and targeted to complement or Fc receptor-bearing APC that activate the Ag-experienced T cells to produce IL-2 rapidly. The combined effects of Ab-facilitated Ag presentation and rapid lymphokine production lead to rapid clonal expansion of the Ag-experienced T cells. However, the poor capacity of these cells to proliferate in response to growth factors limits the magnitude of the expansion. As residual peptide-MHC molecules derived from the primary immunization disappear, the Ag-experienced T cells revert to the naive surface phenotype and slower response status of naive T cells. If the host is challenged with Ag during this period, Ag-specific Ab, which remains elevated in immune hosts for life, will direct the Ag to APC efficiently and enhance the activation of the Ag-experienced T cells that retain rapid lymphokine production potential but whose proliferation is not longer suppressed by residual Ag. If this scenario is correct, then, as proposed by Bell and colleagues (35), CD4 T cell-mediated protection after the primary immunogen has disappeared may be due to the concerted action of Ag-specific Ab and an expanded population of Ag-experienced T cells that share some functional properties with naive cells.

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