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Induction of diverse T cell memory through antibody-mediated activation in mice

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Recent studies have demonstrated that laboratory mice lack the robust memory T cell repertoire found in mice exposed to nature [1, 2]. This deficit has restricted the ability to study the contribution of a diverse, widespread T cell memory repertoire to global immune responses. Activation of diverse T cell memory subpopulations is required to examine contributions of these cells to immune responses and to develop models that more closely mimic human disease.

Previous *in vitro* studies demonstrated that the 145-2C11 antibody to CD3 ϵ induces heterogeneous memory responses that are indistinguishable from responses in peptide-primed memory T cells [3]. We hypothesized that this antibody would induce an acute infection-like response provoking widespread, diverse T cell activation in inbred laboratory mice and isolated, nonspecific and persistent T cell memory.

We administered 50 μ g anti-CD3 ϵ (Clone 145-2C11, Biolegend, San Diego, CA) activating antibody to 10–12-week-old C57Bl/6 mice. Mice injected with isotype antibody served as controls. As previously described [4], treatment reduced total number of splenic CD8 T cells (Fig. 1A). The reduction was significant on day 5 in naïve (CD44⁻/CD11a⁻) CD4 and on days 1 and 5 in naïve CD8 T cells (Fig. 1B, lower panel). Numbers of memory (CD44⁺/CD11a⁺) CD8 T cells increased on day 5 (Fig. 1B, upper panel).

Inoculation also increased (1) percentage of cells expressing CD69 (Fig. 1C; Supporting Information Fig. 1A), (2) level of expression of CD4 and CD8 co-receptors (Fig. 1D; Supporting Information Fig. S1A) [5], (3) percentage of CD8 T cells expressing granzyme B (Fig. 1E), and (4) percentage of CD4 and CD8 T cells expressing Ki67 (Fig. 1F) on day 1.

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The detailed Materials and methods for Letters to the Editor are available online in the Supporting information

Expression of all but Ki67 returned to baseline by day 5. This response indicated acute differentiation, followed by contraction [1].

High doses of anti-CD3e can be fatal [6]. However, no deaths occurred following administration of one-fourth the reported lethal dose. In addition, while total splenic neutrophil counts were higher 24 h after inoculation, these returned to baseline by day 35 (Fig. 1G). No other differences in the innate immune system were detected.

Figure 1H depicts effects of inoculation on T cell subsets. The percentage of acute effector (T_{EFF} , $CD44^{-}/CD62L^{-}$) CD4 (7–45%) and CD8 T cells (5–38%) rose dramatically on day 1. These changes were accompanied by a decrease in percentage of naïve ($CD44^{-}/CD62L^{+}$) CD4 (71% to 24%) and CD8 T cells (74 to 30%). No changes in central memory (T_{CM} , $CD44^{+}/CD62L^{+}$) or effector memory (T_{EM} , $CD44^{+}/CD62L^{-}$) CD4 T cells were detected. However, the percentage of CD8 T_{CM} was decreased (18 to 14%) while that of CD8 T_{EM} increased (3.2% to 18%) (Fig. 1H). Examination of total splenic T cells by $CD44/CD62L$ demonstrated a decrease in naïve CD4 and CD8 T cells and an increase in both CD4 and CD8 T_{EFF} by post-inoculation day 1. CD4 T_{EM}/T_{CM} were unchanged by treatment on day 1, while CD8 T_{EM} were increased and T_{CM} were decreased (Supporting Information Fig. S1B).

One day after anti-CD3e treatment the percentages of cells that produced IFN- γ , IL-4, TNF- α , and IL-17- α were higher than pre-treatment, indicating *in vivo* activation (Fig. 1I; Supporting Information Fig. S1C).

To identify long-term effects of inoculation on the T cell repertoire, we compared anti-CD3e treated mice to controls at 35 days following inoculation [7]. No differences in total number of splenic T cells or total splenic CD4 T cells were observed (Fig. 2A). The number of CD8 T cells was lower in anti-CD3e treated mice (Fig. 2A). No difference in total of CD8 T cells in liver or lungs were noted but the number of hepatic CD4 T cells was reduced in anti-CD3e treated mice (Supporting Information Fig. S2A). Anti-CD3e treatment altered splenic T cell subpopulations. Specifically, total number and fraction of splenic memory ($CD44^{+}/CD11a^{+}$) T cells was higher in anti-CD3e treated mice (Fig. 2B, left; Supporting Information Fig. S2B). The number of naïve ($CD44^{-}/CD11a^{-}$) T cells in anti-CD3e treated mice was correspondingly lower (Fig. 2B, right; Supporting Information Fig. S2B). The percentage of T_{EM}/T_{CM} CD4 T cells in spleens isolated from anti-CD3e treated mice was higher than in controls (38% vs. 29%); similar differences were noted in CD8 T_{EM} (19% vs. 10%) and T_{CM} (33% vs. 22%) populations. Further, percentages of naïve T cells from anti-CD3e treated animals were lower than in controls (Fig. 2C). While anti-CD3e treatment increased the fraction of memory T cells, the total numbers of splenic T cell numbers also reflected a memory phenotype (Supporting Information Fig. S2C).

The percentage of CD4 T_{CM} and CD8 T_{EM} in the liver was higher in anti-CD3e treated mice. Reciprocal changes were noted in naïve CD8 T cells (Supporting Information Fig. S2D). In the lung, the percentage of CD4 T_{CM} and CD8 T_{EM} was higher in anti-CD3e treated mice (Supporting Information Fig. S2D).

Anti-CD3 ϵ treatment was characterized by a lower percentage of CD4 T regulatory cells (CD25⁺/FoxP3⁺) (Fig. 2D). Finally, relative to controls, anti-CD3 ϵ treated mice displayed a greater percentage of CD44⁺/CD127⁺ CD4 and CD8 T cells (Fig. 2E) further indicating effective memory induction.

To identify global changes in T cells, we examined pooled CD4 and CD8 T cells from control and anti-CD3 ϵ treated mice globally using t-distributed stochastic neighbor embedding of 11 T cell markers (Fig. 2F, markers listed in methods). Areas consistent with T cell memory (CD44⁺/CD11⁺) demonstrate increased density on pseudocolor plots in anti-CD3 ϵ treated mice while naïve T cells (CD44⁻/CD11a⁻) were enriched in control animals, demonstrating global change due to anti-CD3 ϵ . Similarly, a greater percentage of CD8 T cells from anti-CD3 ϵ treated mice responded to stimulation by producing IL-2 and IFN- γ (Fig. 2G). Finally, T_{EM}/T_{CM} (CD44⁺) were increased in circulating T cells isolated from mice 135 days after inoculation (Fig. 2H).

Treatment with anti-CD3 ϵ antibody led to widespread, effective activation of both CD4 and CD8 T cells resulting in persistent long-term T cell memory formation and induction of a broad memory T cell repertoire. This approach enables *in vivo* study of isolated T cell memory. Induction T cell memory without issues presented by pet store mice addresses an essential differences between mice and men. This approach may provide a more effective model of human disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Effects of anti-CD3e treatment at 24 and 120 h.

Mice received 50 μ g of anti-CD3e or control antibody. Animals euthanized 24 or 120 h later. Circles, isotype treated control; squares, day 1; diamonds, day 5. Splenic cells analyzed by flow cytometry. (A) Total T cells. (B) Memory (CD44⁺/CD11a⁺, top) and naïve (CD44⁻/CD11a⁻, bottom) T cells. (C) Percent CD69 positive. (D) Median fluorescence intensity (MFI) of CD4 and CD8 on respective cells. (E) Percent granzyme B⁺ CD8 T cells. (F) Percent Ki67⁺ T cells. (G) Total neutrophils at 24 h and 35 days. (H) Plots of CD4 (Top) or CD8 (Bottom) T cells following treatment. (I) IFN- γ and TNF- α production by T cells. Gating in Supporting Information Figs. S3 and S4. Significance determined using one-way ANOVA, * p < 0.05, ** p < 0.01, *** p < 0.001. Data as mean+SD, pooled from two to three experiments except (D), N = 3–6 mice/group.

Figure 2. Long-term effects of treatment with anti-CD3 ϵ activating antibody.

Mice received either intravenous dosing of anti-CD3 ϵ (50 μ g) or isotype antibody. Animals sacrificed 35 days later. Circles, control; squares, anti-CD3 ϵ mice. (A) Total, total CD4, and total CD8 T cells. (B) Total splenic memory (CD44⁺/CD11a⁺) and naïve (CD44⁻/CD11a⁻) T cells. (C) Plots (CD44/CD62L) of splenic T cells from control and anti-CD3 ϵ mice. (D) Percent splenic CD25⁺/FoxP3⁺ T cells. (E) Percent splenic CD127⁺/CD44⁺ T cells. (F) Pseudocolor tSNE of isotype or anti-CD3 ϵ treated splenic T cells. Key demonstrates memory (CD44⁺/CD11a⁺, Red, CD4; Orange, CD8) or naïve (CD44⁻/CD11a⁻, Blue, CD4; Aqua, CD8) T cells. (G) Effect of PMA/Ionomycin stimulation on IFN- γ , IL-2, and TNF- α production. (H) Percent memory (CD44⁺) T cells in peripheral blood 135 days post-treatment. Gating in Supp. Fig. 3 and 4. Significance determined using two-tailed *T*-test, **p* < 0.05, ***p* < 0.01. Data pooled from two to three experiments, shown as mean + SD, *N* = 4–6 mice/group.