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Complete but curtailed T cell response to very low affinity antigen

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Abstract

Following an infection, CD8⁺ T cells are activated and undergo a characteristic kinetic sequence of rapid expansion, subsequent contraction and formation of memory cells^{1–3}. The pool of naïve T cell clones is diverse and contains cells bearing T cell antigen receptors (TCR) that differ in their affinity for the same antigen^{4,5}. How these differences in affinity impact the function and the response kinetics of individual T cell clones was previously unknown. Here we show that during the *in vivo* response to microbial infection, even very weak TCR-ligand interactions are sufficient to activate naïve T cells, induce rapid initial proliferation and generate effector and memory cells. The strength of the TCR-ligand interaction critically impacts when expansion stops, when the cells exit lymphoid organs and when contraction begins, *i.e.* strongly stimulated T cells contract and exit lymphoid organs later than do weakly stimulated cells. Our data challenges the prevailing view that strong TCR ligation is a prerequisite for CD8⁺ T cell activation. Instead, very weak interactions are sufficient for activation, but strong TCR ligation is required to sustain T cell expansion. We propose that in response to microbial challenge, T cell clones with a broad range of avidities for foreign ligands are initially recruited, and that the pool of T cells subsequently matures in affinity due to the more prolonged expansion of high affinity T cell clones.

To date our picture of the kinetics of the CD8⁺ T cell response to pathogens comes from analysing the response of monoclonal TCR transgenic T cells to high affinity TCR ligands^{1–3,6–11}. We set up experiments to compare the polyclonal response of endogenous T cells with that of TCR transgenic T cells at early and late time points following an infection. 3×10^3 OT-1 TCR transgenic CD8⁺ T cells, specific for an ovalbumin (Ova) peptide plus H-2K^b, were transferred into mice that were subsequently infected with recombinant *Listeria monocytogenes* expressing Ova (Lm-N4ova). On day 4 post infection (*p.i.*) the mice contained similar numbers of K^b/Ova-specific endogenous CD8⁺ cells and OT-1 T cells (Fig. 1A,B). In contrast, by day 7 the ratio had shifted by more than 30-fold in favour of the OT-1 cells (Fig. 1A,B). This shift in the ratio indicated that the OT-1 cells expanded between day 4 and 7 better than the endogenous T cells. The population of endogenous

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CD8⁺ T cells on day 4 was far less sensitive to antigen than were the OT-1 cells (Fig. 1C) but by day 7 both populations were equally sensitive to antigen stimulation (Fig. 1D). In addition, we detected changes in the TCR β chain usage among endogenous K^b/Ova specific T cells between day 4.5 and 7.5 p.i. (Supp. Fig. 1). Thus, while many T cells with low functional avidity are initially recruited into the response, higher avidity T cells expand better than lower avidity clones, suggesting a more complex dynamic within the endogenous population than a simple equal and synchronous expansion of all the recruited clones.

Low functional avidity can occur when T cells carry a TCR that recognises a ligand with low affinity¹². To study how TCR affinity for antigen impacts clonal propagation in a controlled situation, we chose a model in which TCR transgenic OT-1 cells are stimulated by different altered peptide ligands (APL). We selected five APL derived from the original OT-1 ligand SIINFEKL (N4). The APL bind equally well to H-2K^b as N4 but differ in their potency of stimulating OT-1 cells (Supp. Fig. 2). To ensure that the APL are presented to OT-1 cells in a physiological context during an infection we generated recombinant *Listeria monocytogenes* strains engineered to express chicken Ova protein containing APL (Lm-APLova) in place of the N4 epitope.

All of the Lm-APLova strains, even Lm-V4ova, containing the V4 peptide that is about 700-fold less potent than N4 (Supp. Fig. 2), expand OT-1 cells in vivo (Supp. Fig. 3). Nonetheless, there was a direct correlation between OT-1 numbers and the affinity of the priming ligand on day 6 p.i. (Fig. 2A). To study the initial kinetics of the OT-1 response to Lm-APLova, we grafted mice with higher numbers of CFSE labeled OT-1 cells and infected them with *Listeria* expressing either the native N4 peptide, or the low affinity APL, Q4, or the very low affinity APL, V4. In all cases, at 78 hours p.i., the majority of divided OT-1 cells are in their 6th and 7th division (Fig. 2B). This indicates that the affinity of TCR interaction does not dictate the rate of initial cell division. Lm-N4ova and Lm-Q4ova drive similar accumulation of OT-1 T cells in the spleen 4 days p.i. (Fig. 2C). However, while the N4 stimulated OT-1 cells keep expanding and plateau at day 7 p.i., Q4 stimulated OT-1 reach their maximum number on day 5.5 and start to contract earlier than N4 stimulated OT-1 (Fig. 2C). Cells stimulated by weaker ligands showed reduced BrdU incorporation at day 5.5 p.i. compared to N4 primed OT-1 cells implying that they go through fewer cell divisions rather than dividing and dying (Supp. Fig. 4). Measuring the frequency of OT-1 cells after infection with each of the different Lm-APLova strains revealed that the weaker the ligand, the earlier the cells reach their maximum level of expansion and begin to contract (Fig. 2D). Thus, while low affinity ligands initiate the same rate of rapid proliferation as high affinity ligands, the final magnitude of expansion and the timing of the onset of contraction are determined by the strength of the TCR-ligand interaction. The efficient recruitment of low affinity CD8⁺ T cells and their shorter expansion period explain the data presented in Fig. 1.

T cells stimulated by low affinity ligands, including V4, were phenotypically similar to cells stimulated by high affinity ligands in terms of expression of CD11b, CD27, CD28, CD44, CD122 and PD-1 on days 4 and 7 p.i. (data not shown). OT-1 cells stimulated by low and high affinity ligands expressed Granzyme B on day 4 p.i., lysed antigen positive target cells in vivo and produced IFN γ and TNF α in response to peptide stimulation on day 7 p.i. (Supp.

Fig. 5). The only major phenotypic differences we observed between OT-1 cells stimulated by low and high affinity ligands were the surface levels of CD25 and CCR7, which were lower on weakly stimulated OT-1 cells on day 4 p.i. (Fig. 2E). These data reveal that even very weak TCR ligation induces cells with typical effector phenotypes.

Following *Listeria* infection, specific CD8⁺ T cells meet antigen in the splenic periarteriolar lymphocyte sheaths (PALS) and undergo explosive proliferation before any progeny exit the PALS and enter the red pulp and the blood stream^{13,14}. Supplemental Figure 6 shows that the progeny of a single OT-1 cell stimulated by Lm-N4ova remains in the same PALS until day 4. Surprisingly, OT-1 cells stimulated by weak ligands such as Q4 and V4 can be detected in the blood and in the red pulp at 4 days p.i., before N4 stimulated OT-1 cells appear in these compartments (Fig. 3). Similarly, in the polyclonal endogenous response, effector T cells detectable in the blood at day 4.5 p.i. are of much lower functional avidity than the cells found at day 7.5 (Supp. Fig. 7). The early appearance of weakly stimulated cells in the blood and the red pulp correlates with the earlier downregulation of CCR7 on Q4 stimulated OT-1 (Fig. 2E). These data show that ligand affinity dictates the kinetics of T cell migration and that, remarkably, the earliest wave of effectors released into the blood stream is dominated by T cells bearing low affinity TCR.

All of the APL also induce an endogenous CD8⁺ T cell response (data not shown). In the case of APL that interact weakly with the OT-1 TCR, some endogenous CD8⁺ T cell clones respond better to the APL than do the OT-1 cells (data not shown). Could competition with endogenous T cells bearing higher affinity TCR explain the early migration out of the spleen and the shortened expansion time of weakly stimulated OT-1 cells? Speaking against this notion, we found that the response to Lm-Q4ova or Lm-V4ova was not enhanced in TCR $\alpha^{-/-}$ P14 TCR transgenic mice which completely lack an endogenous CD8⁺ T cell response to Q4 or V4 (Supp. Fig. 8).

We went on to ask whether CD8⁺ T cells stimulated by low affinity ligands converted to memory cells. We followed the response of OT-1 cells stimulated by the different ligands for 138 days and, in all cases, detected a population of memory OT-1 cells that remained stable in numbers beyond day 21 (Fig. 4A,B). When challenged with recombinant vesicular stomatitis virus expressing Ova (VSV-N4ova) the OT-1 cells previously exposed to any of the APL, expanded significantly (Fig. 4C). Control mice that initially received a wildtype *Listeria* infection, contained far fewer OT-1 cells after VSV-N4ova challenge, indicating that all the APL generated memory cells. These results were confirmed using Rag2^{-/-} OT-1 cells (data not shown). When N4 and V4 memory OT-1 cells were transferred into new hosts, both expanded comparably after Lm-N4ova rechallenge. Notably, both memory populations also responded similarly Lm-V4ova rechallenge, albeit the latter response was reduced in magnitude compared to Lm-N4ova (Supp. Fig. 9). Thus, expansion in the recall response is determined by the strength of the recall stimulus and not by the priming antigen. These data, showing that even very weak TCR-ligand interaction drives the formation of functional memory T cells, are in line with reports indicating that lymphopenia-driven homeostatic expansion, during which T cells also encounter only very weak TCR ligands, generates functional memory T cells¹⁵.

The functional avidity of T cell clones can change over the course of an immune response^{16,17}. In our experiments OT-1 T cells serve as an internal control for the changes in functional avidity at the clonal level. As such changes affect both endogenous and TCR transgenic T cells, the shift in the avidity of endogenous T cells compared to OT-1 T cells reflects changes in the T cell repertoire. Our data therefore show that microbial challenge recruits CD8⁺ T cell clones with an extremely broad range of functional avidities for foreign antigen and that the composition of the initial repertoire rapidly changes to favour the “best fit”, high avidity T cells. This evolution of the repertoire is explained by the fact that very low affinity TCR ligands are sufficient to induce rapid proliferation, but the length of the expansion period and the burst size directly correlate with the strength of TCR ligation so that cells stimulated by strong agonists go through more divisions than cells stimulated by weak agonists. Some viral pathogens can readily mutate their antigenic motifs^{18,19}. The recruitment of a broad repertoire raises the possibility that some of the low avidity effector and memory cells may be advantageous in controlling these pathogen variants²⁰.

TCR ligands of different affinity induce opposing cell fates during thymocyte development i.e. positive versus negative selection^{21–23}. The peptides we incorporated into *Listeria* to study the response of peripheral OT-1 cells can be directly compared to the APL used by Daniels et al. to study selection of immature OT-1 thymocytes²². The T4 ligand, used in both studies, is on the threshold of positive versus negative selection. The V4 ligand used here is much weaker than T4 (EC₅₀ 700 vs. 71) and induces a much weaker peripheral OT-1 response than T4. Recombinant *Listeria* making the Q4H7 or Q7 ligands, which are closer in potency to T4, yet still capable of positive selection only, induced peripheral OT-1 expansion that was intermediate between T4 and V4 (Supp. Fig. 10). This data raises the possibility that the minimum affinity needed to activate peripheral T cells during an infection overlaps the affinity required for thymocyte positive selection on self-peptides, with implications for autoimmunity. Furthermore, while low versus high affinity ligands induce different fates in thymocytes²³, our data indicate that peripheral CD8⁺ T cells do not show the same sharp ligand discrimination.

Methods

CD45.1 or Thy1.1 congenic OT-1 TCR transgenic naïve T cells were isolated using the CD8 untouched isolation kit (Miltenyi Biotech) plus biotin conjugated anti-CD44. Cells were transferred into C57/BL6 (Jackson Laboratories) mice 1 day prior to infection.

Recombinant *Listeria monocytogenes* strains were generated that stably express chicken Ovalbumin (AA_{134–387}) containing either the native ligand SIINFEKL_{257–264} or the APL listed in Supp. Fig. 2. The designation of the APL indicates the substituted amino acid and the position within the SIINFEKL epitope. A previously described cassette²⁴ encoding for the expression of secreted Ova was manipulated by site-directed mutagenesis to insert the APL. The cassettes were cloned into a vector (**pPL2**) and stable *Listeria* recombinants were made as described²⁵. Mice were infected i.v. with 1000 CFU of log phase *Listeria*. For VSV-N4ova, mice were infected i.v. with 2×10⁵ PFU.

Spleens were digested with Blendzyme 2 and DNase 1 (both Roche) for 1 hour at 37°C and mashed through a 100 µm cell strainer (Becton Dickinson) to obtain single cell suspensions. Flow cytometry, intracellular cytokine staining (ICS), and CFSE labeling were performed using standard procedures. For ICS, the cells were first stimulated at 37°C for 30 min with peptide, then 7 µM Brefeldin was added and the incubation was continued for another 4.5 hours. For CCR7 staining, cells were incubated for 1 hour at 37°C and then stained on ice with CCL19-IgG fusion supernatant²⁶. To assess the level of non-specific binding of CCL19-IgG to OT-1, soluble CCL19 (R&D Systems) was added to control samples.

For immunofluorescent microscopy, spleens were fixed in 4% paraformaldehyde and kept in 30% sucrose over night. 10–20 µm sections, were cut and stained using standard procedures and analysed on a Zeiss 510 META confocal microscope.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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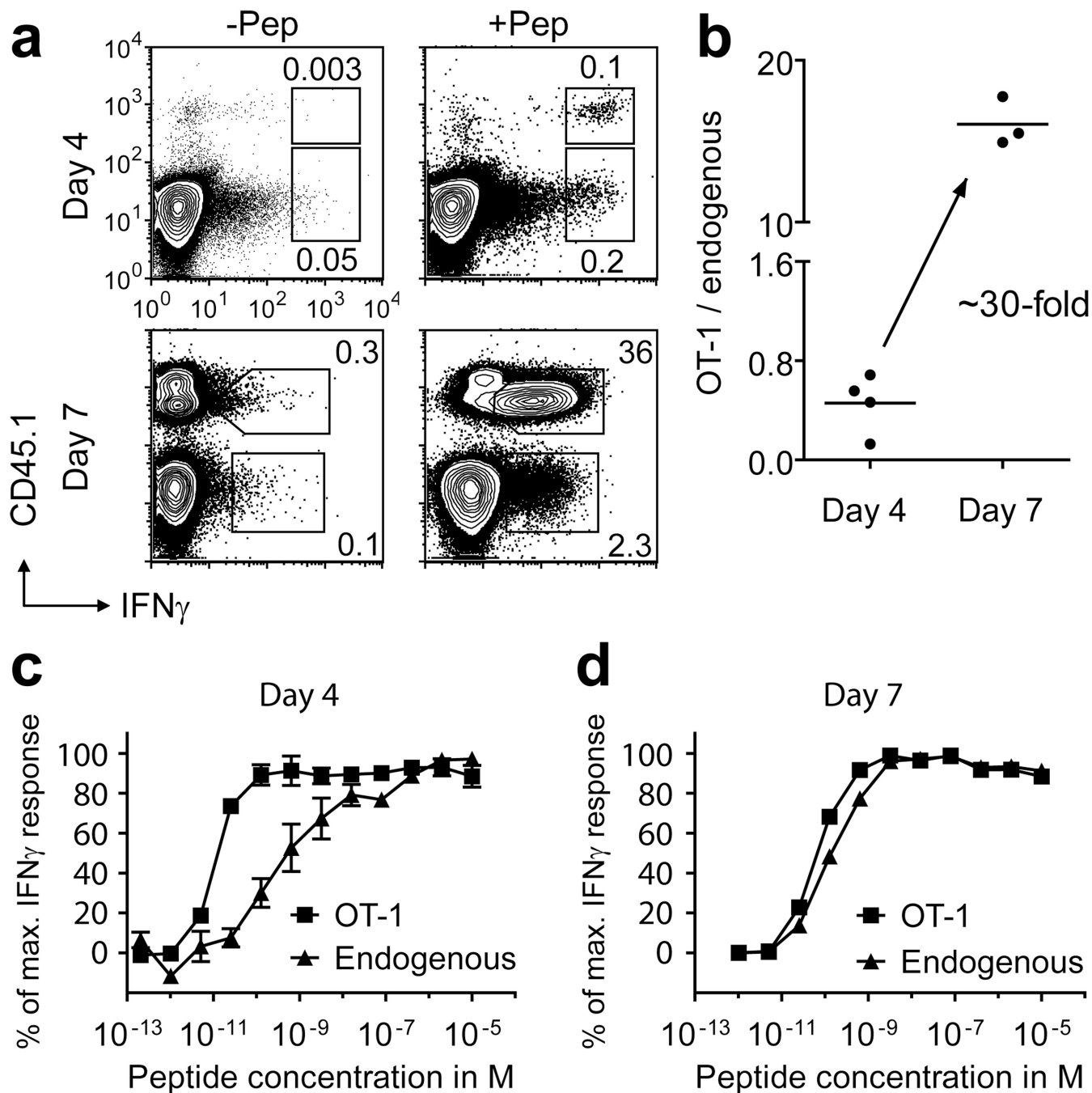


Fig. 1. Unequal propagation of OT-1 and endogenous CD8⁺ T cells

C57/BL6 mice were grafted with 3×10^3 naive CD45.1 OT-1 cells and infected with *Listeria monocytogenes* expressing Ova. On days 4 and 7 p.i., total splenocytes were harvested and briefly re-stimulated with Ova peptide in vitro. **A**, Representative CD8⁺ gated flow plots of splenocytes stimulated with or without 10 μ M peptide are shown. Frequencies refer to total CD8⁺ cells. **B**, The ratios of the numbers of OT-1 and endogenous IFN γ ⁺ T cells found in individual mice are graphically presented. **C** and **D**, Peptide dose-response curves

normalized to the level of maximum numbers of IFN γ producing OT-1 and endogenous K^b/Ova-specific T cells are depicted (N=4 day 4, N=3 day 7), bars show standard error.

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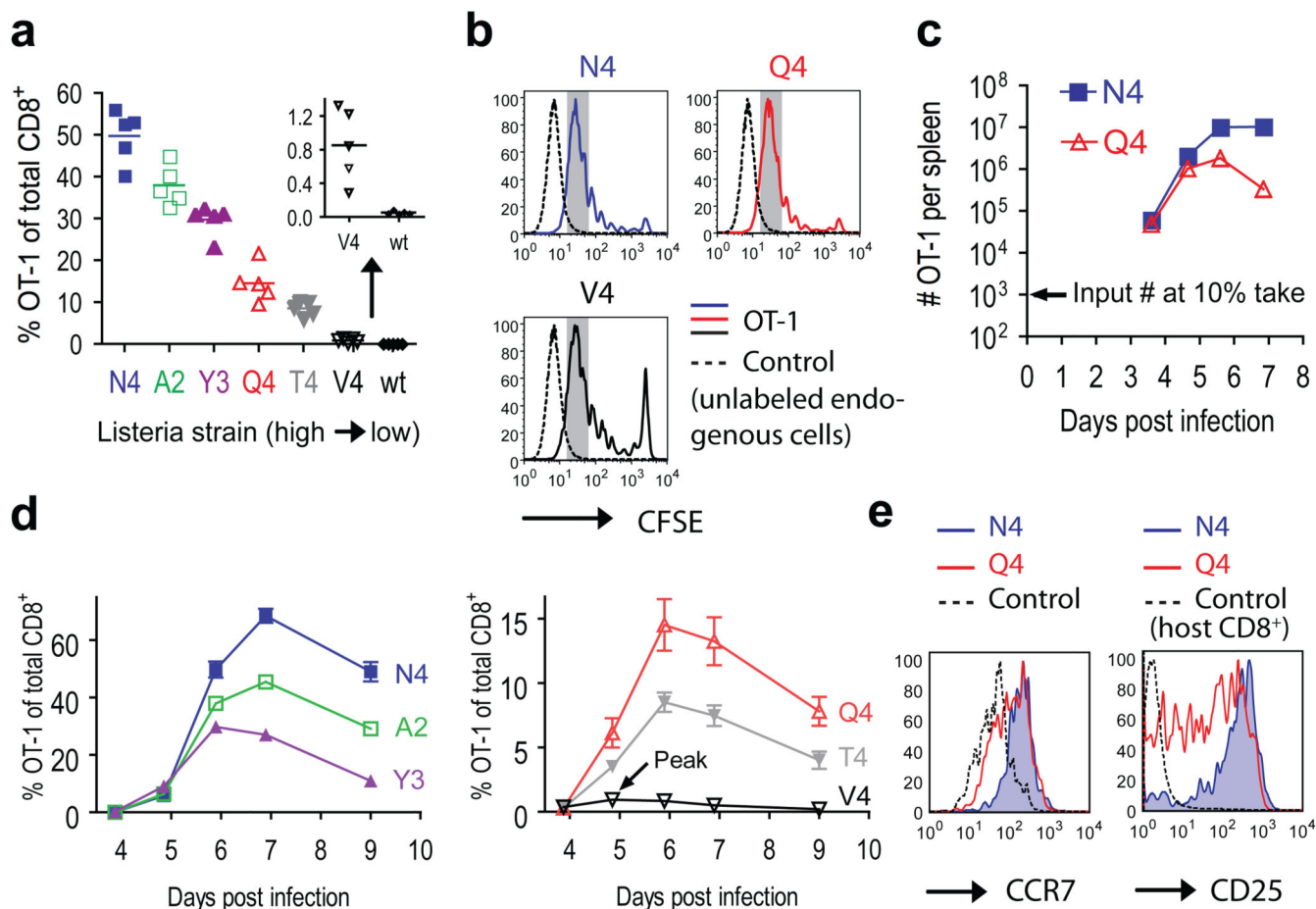


Fig. 2. The strength of TCR ligation dictates the timing of T cell contraction

Mice were grafted with 10^4 unlabeled (in **A**, **C**, **D**, **E**) or 2×10^5 CFSE labeled naive OT-1 cells (**B**) and infected with wildtype (wt) or recombinant *Listeria monocytogenes* expressing Ova protein containing native SIINFEKL (N4) or the indicated altered peptide ligands (APL), listed in order of decreasing potency (Supp. Fig. 2). **A**, The frequency of OT-1 among CD8⁺ cells in the blood at day 6 p.i., the insert shows Lm-V4 and wt Listeria on a magnified scale; **B**, CFSE dilution profiles of splenic OT-1 cells at day 3.25 p.i.; **C**, Numbers of splenic OT-1 cells at the indicated time points (N=4 per group and timepoint); **D**, Frequency of OT-1 among CD8⁺ blood cells at the indicated time points (N=5), bars show standard error; and **E**, Surface CCR7 and CD25 levels on splenic OT-1 at day 4 p.i.

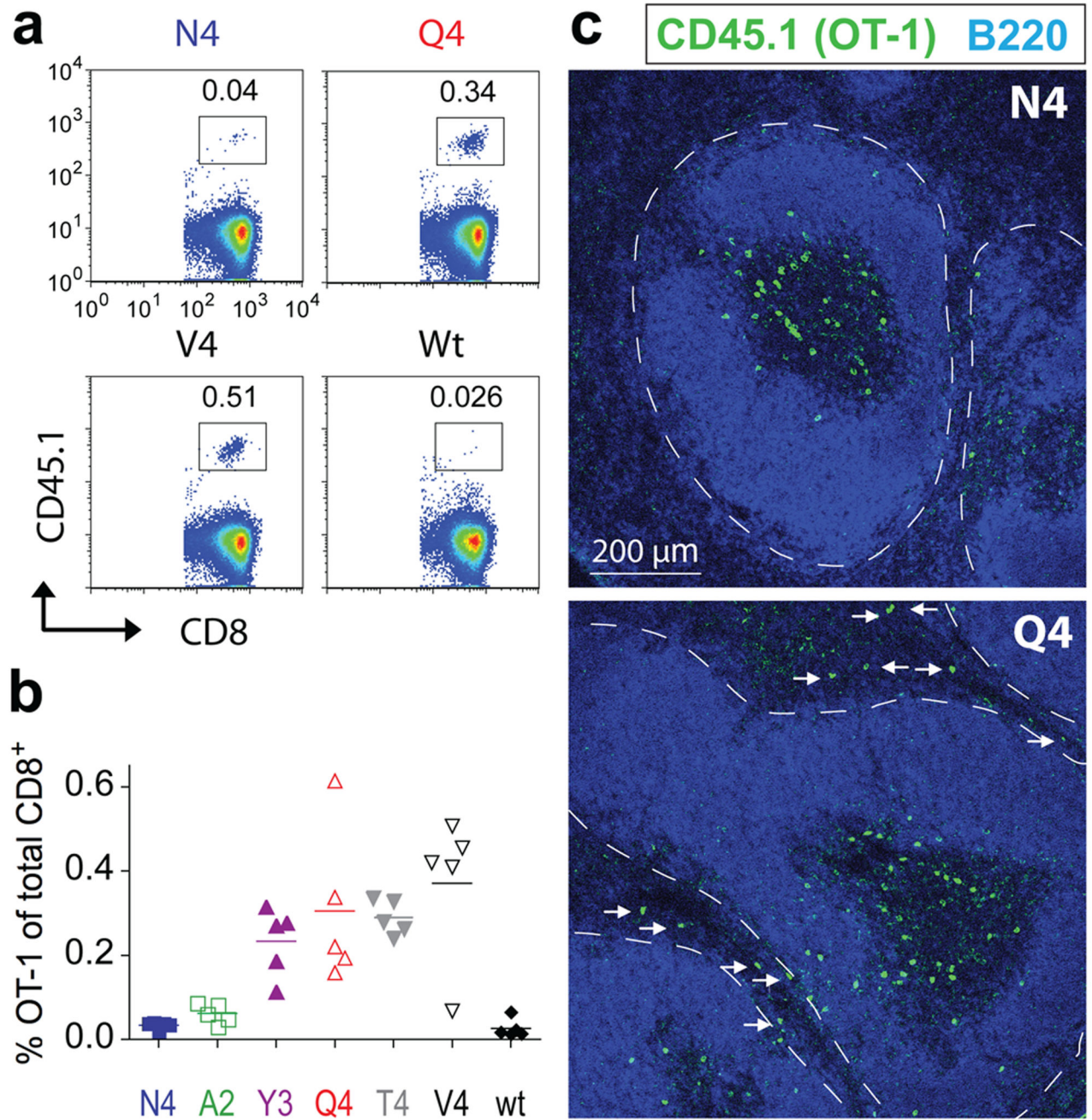


Fig. 3. The strength of TCR ligation determines migration kinetics

10^4 (A,B) and 3×10^3 naïve OT-1 cells (C) were transferred into C57/BL6 mice, which were subsequently infected with Lm-N4ova or Lm-APLova strains. A, Representative flow plots of CD8⁺ gated white blood cells showing the frequency of OT-1 cells at 4 days p.i. B, shows data for all APL (N=5). C, Splenic sections taken at day 4 p.i. show the distribution of OT-1 cells stimulated by N4 or Q4. OT-1 cells are green and B cells are blue. The border of PALS and red pulp is marked and arrows indicate OT-1 cells within the red pulp.

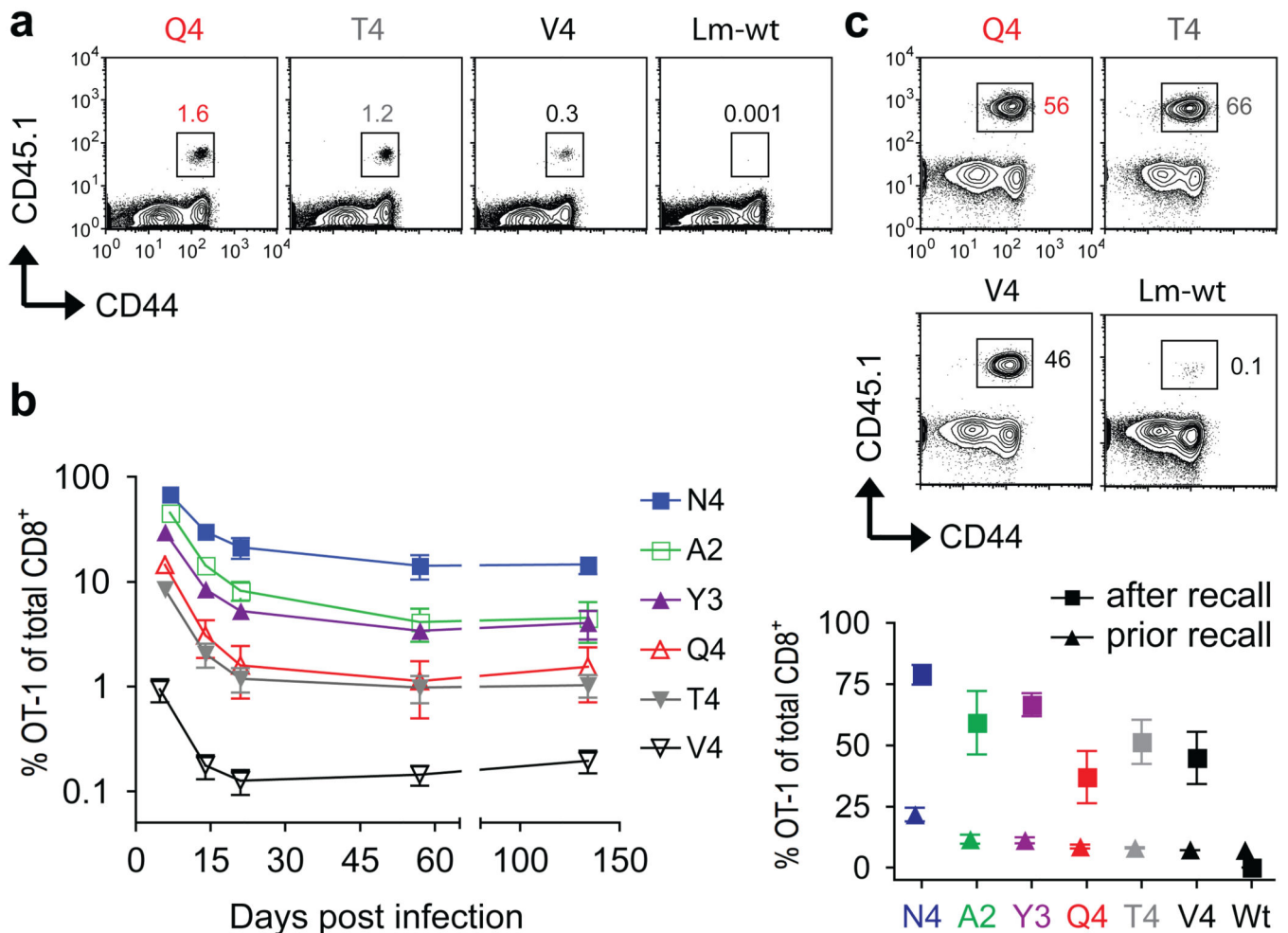


Fig. 4. Low potency TCR ligands induce functional memory cells

Mice were grafted with congenic naïve OT-1 cells and infected with Lm-wt, Lm-N4ova or Lm-APLova strains. **A**, Representative flow plots of the frequencies of memory OT-1 among blood CD8⁺ T cells at day 138 p.i. **B**, the contraction and memory kinetics for all mice and all APL (N=5) until day 138 p.i. **C**, Mice were rechallenged on day 138 p.i. with VSV-N4ova and the frequencies of OT-1 among blood CD8⁺ T cells were determined 4 days later. The upper panels show representative flow plots and the lower depicts the data for all mice (N=5), with triangles indicating the frequency before and squares the frequency after the recall. Bars show standard error.