ARTICLES

Restoring function in exhausted CD8 T cells during chronic viral infection

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Functional impairment of antigen-specific T cells is a defining characteristic of many chronic infections, but the underlying mechanisms of T-cell dysfunction are not well understood. To address this question, we analysed genes expressed in functionally impaired virus-specific CD8 T cells present in mice chronically infected with lymphocytic choriomeningitis virus (LCMV), and compared these with the gene profile of functional memory CD8 T cells. Here we report that PD-1 (programmed death 1; also known as Pdcd1) was selectively upregulated by the exhausted T cells, and that *in vivo* administration of antibodies that blocked the interaction of this inhibitory receptor with its ligand, PD-L1 (also known as B7-H1), enhanced T-cell responses. Notably, we found that even in persistently infected mice that were lacking CD4 T-cell help, blockade of the PD-1/PD-L1 inhibitory pathway had a beneficial effect on the 'helpless' CD8 T cells, restoring their ability to undergo proliferation, secrete cytokines, kill infected cells and decrease viral load. Blockade of the CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) inhibitory pathway had no effect on either T-cell function or viral control. These studies identify a specific mechanism of T-cell exhaustion and define a potentially effective immunological strategy for the treatment of chronic viral infections.

Memory CD8 T-cell differentiation proceeds along distinct pathways after an acute versus a chronic viral infection[1–3]. Memory CD8 T cells generated after an acute viral infection are highly functional and constitute an important component of protective immunity. In contrast, chronic infections are often characterized by varying degrees of functional impairment of virus-specific T-cell responses, and this defect is a principal reason for the inability of the host to eliminate the persisting pathogen. Although functional effector T cells are initially generated during the early stages of infection, they gradually lose function during the course of the chronic infection. This exhaustion of virus-specific T cells was first shown during persistent LCMV infection of mice[4,5]. However, these findings were quickly extended to other model systems, as well as to chronic infections in humans, in particular human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) infections—three chronic infections afflicting >500 million people worldwide[6–8].

PD-1 is upregulated during chronic viral infection

To examine the mechanism of T-cell dysfunction during chronic infection, we used the mouse model of infection with LCMV. An advantage of this model is the availability of LCMV strains that can cause either acute or chronic infections in adult mice; the Armstrong strain is cleared within a week, whereas the clone 13 strain establishes a persistent infection[3]. As these two strains differ in only two amino acids in the entire genome[9], and neither of these mutations affects any of the known T-cell epitopes, it is possible to track the same CD8 T cell responses after an acute or chronic viral infection. In contrast to the highly robust memory CD8 T cells generated after an acute Armstrong infection, LCMV-specific CD8 T cells become exhausted during a persistent clone 13 infection[3–5]. An example of impaired cytokine production and proliferation by the exhausted CD8 T cells is shown in Fig. 1a. As a first step towards understanding the mechanisms of T-cell dysfunction, we performed a comparative genome-wide microarray analysis (Affymetrix) of genes expressed by exhausted versus functional LCMV-specific CD8 T cells of the same antigenic specificity. The most notable finding was the pronounced upregulation of messenger RNA encoding PD-1, an inhibitory receptor of the CD28 family[10–13], by the exhausted LCMV-specific CD8 T cells (Fig. 1b). This was confirmed at the protein level using antibodies specific for PD-1 (Fig. 1b). We found that high expression of this inhibitory receptor was a signature of the functionally exhausted CD8 T cells; all LCMV-specific CD8 T cells (comprising multiple epitopes) that were present in chronically infected mice expressed high levels of PD-1, whereas functional LCMV-specific memory CD8 T cells of the same antigenic specificities present in mice that had cleared the acute LCMV infection did not express any detectable levels of PD-1 (Fig. 1b and data not shown).

We next analysed the kinetics of PD-1 expression during both acute and chronic LCMV infection, and found that PD-1 was transiently expressed on early effector CD8 T cells after LCMV Armstrong infection but then was rapidly downregulated (Fig. 1c). In contrast, PD-1 expression continued to increase on virus-specific CD8 T cells in chronically infected mice and the high level of expression was sustained. PD-1 has two ligands—PD-L1 (B7-H1)[14,15], which is widely expressed on both haematopoetic and parenchymal cells, and PD-L2 (B7-DC)[12,13], which is predominantly expressed on macrophages and dendritic cells. We found that PD-L1 was expressed at very high levels in splenocytes from persistently infected mice, especially on virally infected cells (Fig. 1c). Thus, not only did the exhausted CD8 T cells express high levels of PD-1, but its

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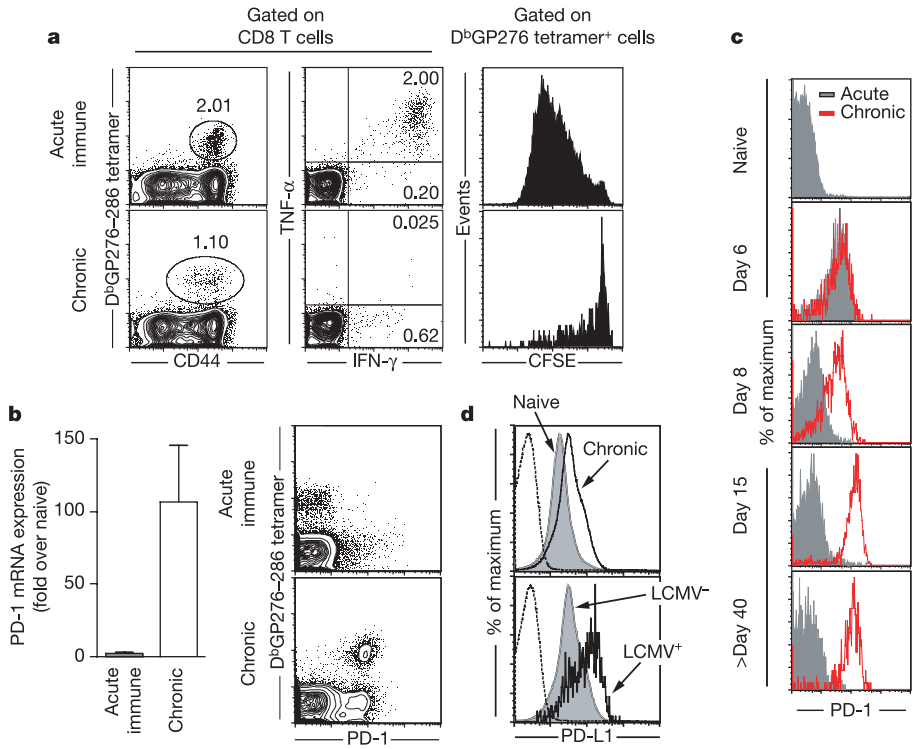

Figure 1 | PD-1 is highly upregulated by exhausted CD8 T cells during chronic infection. **a**, GP276-specific CD8 T cells from LCMV Armstrong immune and clone 13 chronically infected mice (>60 days post-infection) were stimulated with peptide and analysed for cytokine production (5 h) and proliferation (60 h). **b**, PD-1 mRNA (Affymetrix array) and protein expression in virus-specific CD8 T cells isolated from acutely or chronically infected mice (>60 days post-infection). **c**, Kinetics of PD-1 expression on D^bGP276–286 tetramer-positive CD8 T cells during acute (Armstrong) and chronic (clone 13) infection. The top plot is gated on CD8 T cells from a naive mouse. **d**, PD-L1 surface expression on splenocytes from naive and chronically infected mice (top plot) and on LCMV-infected or uninfected splenocytes from a chronically infected mouse (bottom plot). Error bars are s.e.m. throughout. Numbers on FACS plots indicate the percentage of cells in each region.

ligand was also upregulated on infected cells, suggesting a role for this inhibitory pathway in regulating T-cell function during chronic LCMV infection.

PD-L1 blockade enhances viral control and T-cell responses

To test the above hypothesis, we treated persistently infected mice with anti-PD-L1 blocking antibody and monitored T-cell responses and viral control (Fig. 2). We found that virus-specific CD8 T-cell responses were enhanced both quantitatively and qualitatively by the anti-PD-L1 blockade. The treated mice showed significant increases ($P = 0.02$) in the numbers of LCMV-specific CD8 T cells, as measured by three different MHC class I tetramers (Fig. 2a). Most notably, the T cells became functionally superior following anti-PD-L1 treatment, and a higher fraction of the virus-specific CD8 T cells were capable of

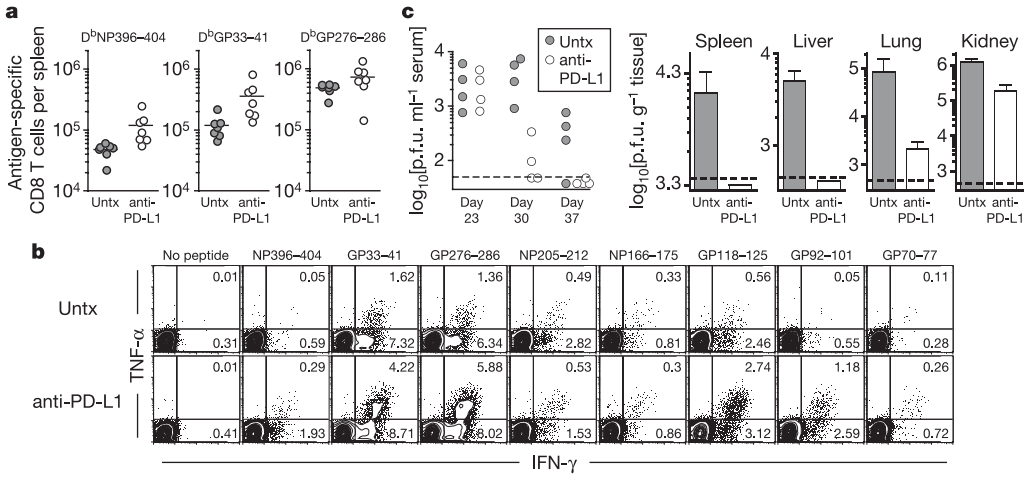

Figure 2 | Blockade of the PD-1/PD-L1 inhibitory pathway increases CD8 T-cell function and enhances the clearance of chronic viral infection. LCMV clone-13-infected mice were treated with anti-PD-L1 from day 23 to day 37 post-infection. **a**, Virus-specific CD8 T-cell numbers in the spleen. Results are pooled from two experiments (NP396 and GP33; $P = 0.02$). **b**, Cytokine production by splenocytes after stimulation with the indicated peptides. **c**, Viral titres in the serum ($P = 0.0016$ at day 30 and $P = 0.035$ at day 37) and indicated tissues at day 37 (spleen $P = 0.003$, liver $P = 0.0084$, lung $P = 0.0076$, kidney $P < 0.0001$). $n = 4$ mice per group. Data are representative of two experiments. Untx, untreated.

producing both interferon (IFN)-γ and tumour necrosis factor (TNF)-α compared to untreated mice ($P = 0.01$) (Fig. 2b). This improved functionality was seen with CD8 T cells responding to multiple epitopes (Fig. 2b). Significantly, PD-L1 blockade resulted in a substantial reduction in virus levels; the treated mice had cleared infectious virus from the serum, spleen and liver, while untreated mice still harboured high levels of virus in these tissues ($P = 0.01$) (Fig. 2c). Also, the anti-PD-L1-treated mice that resolved the infection remained healthy, and did not exhibit any overt signs of disease.

As PD-1 was transiently upregulated during acute LCMV infection, we treated Armstrong-infected mice with anti-PD-L1 antibody during the first week of infection and monitored the T-cell response. We found that PD-L1 blockade did not increase virus-specific CD8 T-cell responses in the acute infection model (data not shown). Thus, it appears that the PD-1 inhibitory pathway is particularly important in exhausted T cells.

Restoring function in 'helpless' CD8 T cells

We next asked whether blockade of the PD-1/PD-L1 inhibitory pathway would enhance CD8 T-cell responses in the absence of CD4 T cell help. This is a much more stringent model of chronic infection and the 'helpless' CD8 T cells show even more pronounced functional defects[4,16,17]. Even under these conditions, when CD4 T cell help was lacking, PD-L1 blockade was highly effective in increasing T-cell numbers, improving their quality, and reducing viral load (Fig. 3). There were approximately sevenfold more D^bGP276–286-specific CD8 T cells ($P < 0.0001$), and fourfold more D^bGP33–41-specific cells ($P < 0.0001$) in the spleens of anti-PD-L1-treated mice than untreated control mice (Fig. 3a, b). There was also a substantial increase in virus-specific CD8 T cells in non-lymphoid tissues such as liver and lung and even in intraepithelial lymphocytes (IEL) (Fig. 3c). To determine whether this increase in cell numbers was due to cell division, we measured 5-bromodeoxyuridine (BrdU) incorporation during the period of anti-PD-L1 treatment (Fig. 3d). In untreated mice, the majority (>55%) of D^bGP276–286-specific CD8 T cells did not incorporate any BrdU, while in treated mice, ≥90% of these cells were positive with much higher levels (mean fluorescence intensity) of BrdU incorporation. In addition to the increased number of BrdU-positive cells, we also found an increased number of cells expressing Ki67, a protein associated with cell-cycle progression (19% in untreated versus 60% in anti-PD-L1 treated mice) (Fig. 3d).

We next measured the effect of anti-PD-L1 blockade on the ability of virus-specific CD8 T cells to produce IFN-γ in the absence of CD4 T cell help. There was a substantial increase in the number of IFN-γ-producing cells in the treated mice (Supplementary Fig. 1). To compare the function of CD8 T cells in untreated and anti-PD-L1-treated mice on a per-cell basis, we calculated the percentage of D^bGP276–286-tetramer-positive cells that were capable of producing IFN-γ. In untreated mice, only ~20% of D^bGP276–286-specific CD8 T cells were capable of producing IFN-γ, whereas in anti-PD-L1 treated mice, ≥50% of these CD8 T cells produced IFN-γ ($P < 0.0001$) (Fig. 3e).

During chronic LCMV infection, exhausted virus-specific cytotoxic T lymphocytes (CTLs) are not able to lyse targets in ^{51}Cr-release assays (Fig. 3f and refs 3–5). Direct *ex vivo* lytic ability became detectable, however, after anti-PD-L1 blockade (Fig. 3f). We also measured the ability of virus-specific CD8 T cells to degranulate by monitoring the appearance of lysosomal markers CD107a/b on the cell surface after re-stimulation with peptide. There was a dramatic increase in the number of virus-specific cells capable of degranulating in anti-PD-L1-treated mice (Fig. 3g). To test whether the increase in IFN-γ production and lytic activity corresponded to better viral control, we measured viral titres in the spleen, liver, lung and serum. As shown in Fig. 3h, there were significant reductions in virus levels in the spleen ($P = 0.008$), liver ($P < 0.0001$), lung ($P = 0.0002$) and serum ($P = 0.003$) in the treated mice. Thus, the results shown in Fig. 3 demonstrate that PD-L1 blockade can restore function even in 'helpless' exhausted CD8 T cells.

To more directly assess the role of PD-1 in T-cell dysfunction, we treated chronically infected mice with a blocking antibody against PD-1 itself. We found that PD-1 blockade also restored function in the exhausted CD8 T cells (Supplementary Fig. 2). However, it is worth noting that blockade with anti-PD-L1 antibody was more effective than anti-PD-1 antibody in enhancing T-cell responses in the chronically infected mice. Potential explanations for this finding include blocking efficiency, differential expression of PD-L1 and PD-L2, or the possibility that PD-L2 might provide 'positive' co-stimulatory signals[18,19].

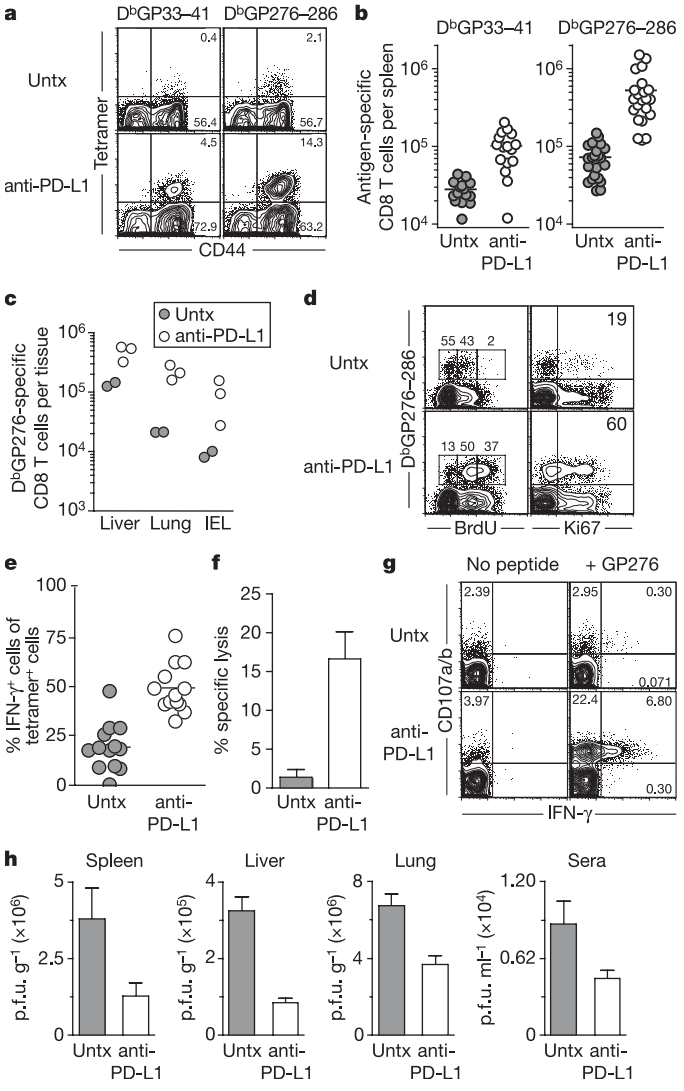

Figure 3 | Restoration of function in 'helpless' exhausted CD8 T cells. Mice were depleted of CD4 T cells and then infected with LCMV clone 13. After at least 40 days, chronically infected mice were treated with anti-PD-L1 for two weeks. Frequency and total number of tetramer-positive CD8 T cells in the spleen (**a**, **b**) or indicated tissues (**c**) ($P < 0.0001$ for GP33 and GP276). **d**, BrdU incorporation and Ki67 expression. $n > 7$ mice per group. **e**, Percentage of D^bGP276–286 tetramer-positive cells capable of producing IFN-γ ($P < 0.0001$). **f**, Direct *ex vivo* ^{51}Cr-release activity using target cells coated with GP33–41, GP276–286 and GP118–125 peptides (effector:target ratio of 100:1). **g**, To measure degranulation by LCMV-specific CD8 T cells, splenocytes were stimulated with peptide in the presence of anti-CD107a/b antibodies and then co-stained for IFN-γ. **h**, Viral titres in the indicated tissues. $n > 10$ mice. (spleen $P = 0.008$, liver $P < 0.0001$, lung $P = 0.0002$, serum $P = 0.03$).

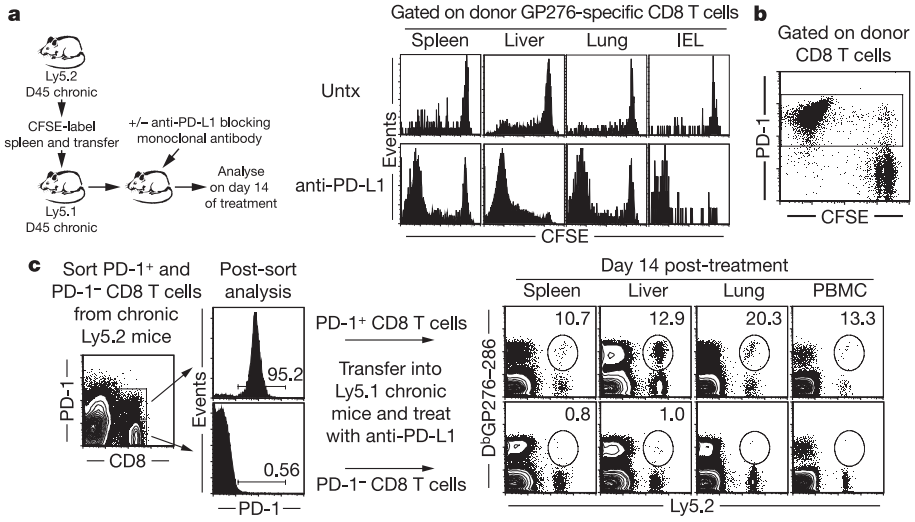

Figure 4 | Proliferation of exhausted CD8 T cells in both lymphoid and non-lymphoid tissues after anti-PD-L1 treatment. a, Ly5.1 and Ly5.2 mice were depleted of CD4 T cells and infected with LCMV clone 13. Splenocytes from chronically infected Ly5.2 mice were CFSE-labelled and transferred into chronically infected Ly5.1 mice. Recipient mice were then treated with anti-PD-L1 for two weeks, and proliferation of virus-specific donor CD8 T cells was analysed in the indicated tissues. **b**, PD-1 expression on proliferating donor CD8 T cells from panel **a**. **c**, PD-1-positive and -negative CD8 T cells from Ly5.2 chronic mice were sorted and transferred into Ly5.1 chronic mice. Mice were then treated with anti-PD-L1 and killed after 14 days. Donor-derived tetramer-positive cells were measured in the indicated tissues. Plots are gated on CD8 T cells. Numbers represent the percentage of donor CD8 T cells specific for $D^bGP276–286$.

CTLA-4 is another inhibitory receptor of the CD28 family, and several studies have shown that treatment with anti-CTLA-4 blocking antibodies can enhance T-cell immunity against tumours[20]. Interestingly, the exhausted LCMV-specific CD8 T cells also expressed elevated levels of CTLA-4 mRNA (data not shown). However, in contrast to our results with anti-PD-L1 or anti-PD-1 antibody blockade, treatment of chronically infected mice with anti-CTLA-4 blocking antibody had no effect on either virus-specific T-cell responses or viral control. Also, there were no synergistic effects of co-blockade with anti-PD-L1 and anti-CTLA-4 (Supplementary Fig. 3).

Proliferation of exhausted CD8 T cells

The expansion of exhausted CD8 T cells was one of the most remarkable effects after blockade of the PD-1/PD-L1 inhibitory pathway. To investigate this further, we used carboxyfluorescein diacetate, succinimidyl ester (CFSE)-labelled splenocytes from chronically infected Ly5.2 mice, transferred them into chronically infected Ly5.1 mice, and then treated the mice with anti-PD-L1 antibody (Fig. 4a). The use of this congenic system (Ly5.1 versus Ly5.2 mice chronically infected with LCMV) allowed us to distinguish between donor and host T cells and directly monitor the proliferation of the CFSE-labelled exhausted virus-specific CD8 T cells. When the exhausted cells (Ly5.2) were transferred back into a chronically infected mouse (Ly5.1), they underwent minimal to no proliferation in any of the tissues examined (spleen, liver, lung or IEL) (Fig. 4a). In contrast, in the presence of anti-PD-L1 blocking antibody, there was substantial proliferation and expansion of the exhausted virus-specific CD8 T cells in all of the tissues analysed (Fig. 4a). Furthermore, many of the donor cells recovered from both lymphoid and non-lymphoid tissues had diluted CFSE to background levels, showing they had divided more than eight times (Fig. 4a). It is worth noting that all exhausted virus-specific CD8 T cells that had undergone this rapid (antigen-driven) proliferation after anti-PD-L1 blockade still expressed high levels of the inhibitory receptor PD-1 (Fig. 4b). This suggests that continuous engagement of PD-L1 with PD-1 was the key event in inhibiting proliferation of the exhausted CD8 T cells. Once this interaction was disrupted by the anti-PD-L1 blocking antibody, the 'brake' was released and the exhausted CD8 T cells could proliferate in response to viral antigen.

Although our data so far make a compelling argument for PD-1/PD-L1 blockade restoring function in exhausted CD8 T cells, there is a possibility that the enhanced immune responses we are seeing in the chronically infected mice after anti-PD-L1 or anti-PD-1 treatment are actually due to the generation of *de novo* T-cell responses from 'naive' CD8 T cells[21]. To address this issue, we sorted PD-1^+ and PD-1^- CD8 T cells from chronically infected Ly5.2 mice, transferred them into chronically infected Ly5.1 mice, and then treated these mice with anti-PD-L1 antibody. We found that anti-PD-L1 treatment only enhanced immune responses of the sorted PD-1^+ CD8 T cells (the cell population containing exhausted cells), and had no effect on PD-1^- CD8 T cells (containing naive cells). These data show unequivocally that PD-L1 blockade restores function in the exhausted CD8 T cells (Fig. 4c).

It was of interest to determine whether the increased numbers of virus-specific CD8 T cells would be sustained after cessation of the anti-PD-L1 treatment. As shown in Supplementary Fig. 4, T-cell numbers remained stable and more functional for several weeks following the transient blockade. This shows that the expanded population of virus-specific CD8 T cells does not suddenly decline after the antibody treatment has been stopped. This is an important point, because it sets the stage for future studies designing intermittent blockade therapy for increasing, in a step-wise manner, the numbers of virus-specific T cells.

Infection of PD-$L1^{-/-}$ mice

A prediction from our antibody blockade experiments is that T cells should not exhaust in PD-$L1^{-/-}$ mice after infection with LCMV clone 13. To test this, we infected PD-$L1^{-/-}$ mice with either Armstrong or clone 13. When PD-$L1^{-/-}$ mice were infected with the LCMV Armstrong strain (acute infection), they behaved just like wild-type mice, producing normal CD8 T cell responses (Fig. 5a) and controlling the infection. In contrast, when PD-$L1^{-/-}$ mice were infected with LCMV clone 13 they died owing to immunopathologic damage. This result tells us two important things: one, that the PD-1/PD-L1 axis operates primarily under conditions of sustained high levels of antigenic stimulation; and second, that the PD-1 inhibitory pathway may have evolved to regulate immune-mediated damage during a persistent infection by turning 'off' the virus-specific T cells. The pathogen ends up taking advantage of this inhibitory pathway to

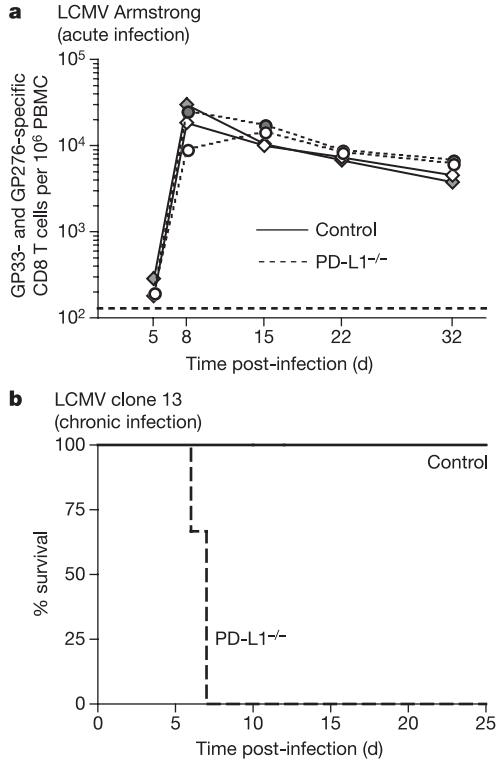

Figure 5 | PD-L1$^{-/-}$ mice make normal CD8 T-cell responses after LCMV Armstrong infection but succumb to clone 13 infection owing to immunopathology. a, PD-L1$^{-/-}$ and littermate control mice were infected with LCMV Armstrong, and virus-specific CD8 T cells were analysed in the peripheral blood mononuclear cells (PBMC) using the tetramers GP33 (diamonds) and GP276 (circles). **b**, PD-L1$^{-/-}$ or littermate control mice were infected with clone 13 and survival was monitored.

establish persistence in the host. However, as we have shown, one can turn the tables on the pathogen by strategically blocking this inhibitory pathway and turning the T cells back on. However, one needs to carefully monitor the potential for autoimmunity and immunopathologic damage under such conditions.

Conclusion

The PD-1 inhibitory pathway is known to regulate immune responses to self antigens[11–13]. Our study now defines a new and critical role for this pathway in modulating T-cell function during chronic viral infection. In addition, we show that PD-1 regulates the distinct pathways of memory CD8 T-cell differentiation observed during acute versus chronic viral infection. PD-1 expression was dramatically upregulated after the effector T-cell stage in chronically infected mice, and was rapidly downregulated in mice that cleared the infection (Fig. 1 and data not shown). It has been suggested that T cells adapt to persistent antigen by downregulating the responsiveness of their T-cell antigen receptor (TCR) signalling machinery[22,23], so defects in virus-specific CD8 T-cell function during chronic infection may be due to inhibition of TCR signal transduction. Inhibition mediated by PD-1 requires close proximity of PD-1 to the site of TCR engagement and does not signal in the absence of a TCR signal. Following crosslinking by PD-1 ligand, the immunoreceptor tyrosine-based switch motif (ITSM) in the cytoplasmic domain of PD-1 is phosphorylated and recruits the phosphatases SHP-1 and SHP-2. These phosphatases act on proximal signalling kinases of the TCR pathway, reducing the TCR signal and leading to diminished T-cell activation and cytokine production[12,13]. Therefore, under conditions of persistent antigen, T cells may modulate their responsiveness by upregulating inhibitory receptors such as PD-1 that attenuate TCR signalling.

Although T-cell dysfunction is a common feature of many chronic viral infections, the underlying mechanisms have remained poorly understood. We have now not only identified a major inhibitory pathway operating during chronic LCMV infection, a classic model of viral persistence in its natural host, but have also developed a simple and highly effective strategy for restoring function in the exhausted CD8 T cells and enhancing viral control. It remains to be determined whether these findings can be extended to other models of chronic infections, and especially to persistent infections of humans. From this perspective, it is worth noting that a study by Iwai *et al.*[24] has shown that PD-1-knockout mice exhibit better control of adenovirus infection, and a recent study[25] using HBV transgenic mice suggests a role for PD-1 in regulating T-cell responses. Thus, it is likely that the immunological strategy we have developed for restoring function in exhausted T cells may have broader applications. Also, our finding that PD-1/PD-L1 blockade was effective in enhancing CD8 T-cell responses even under conditions of CD4 T-cell deficiency is of particular relevance to the situation seen during HIV infection[6,7].

Finally, our studies have implications for therapeutic vaccination and T-cell immunotherapy[26,27]. Despite their enormous potential, therapeutic vaccines have had minimal to no success in eliminating chronic infections. One of the main reasons for their failure is the limited proliferative potential of the exhausted T cells[28]. Our studies now provide a potential strategy for improving the efficacy of therapeutic vaccination by combining it with PD-1/PD-L1 blockade. Similarly, the efficacy of T-cell therapy for chronic infections or tumours may be enhanced by blocking the PD-1 inhibitory pathway.

METHODS

Mice and infections. Ly5.2 C57BL/6, Ly5.1 C57BL/6, 129 PD-L1$^{-/-}$ and 129 littermate control mice were used in this study. LCMV Armstrong and clone 13 infections were performed as described[3]. Where indicated, CD4 T cells were depleted as described[4].

***In vivo* antibody blockade.** Two hundred micrograms of rat anti-mouse PD-L1 (10F.5C5 or 10F.9G2; ref. 29), rat IgG2b isotype control, rat anti-mouse PD-1 (29F.1A12) or anti-mouse CTLA-4 (UC10-4F10-11 or 9D9.1.1.7) were administered intraperitoneally every third day. The two different anti-PD-L1 clones gave indistinguishable results and the isotype control had no effect.

Gene array analysis. Naive P14 transgenic CD8 T cells, D^{b}GP33–41-specific memory CD8 T cells from Armstrong immune mice, and D^{b}GP33–41-specific or D^{b}GP276–286-specific CD8 T cells from CD4-depleted clone-13-infected mice were purified by fluorescence-activated cell sorting (FACS), and RNA isolation and gene array analysis was performed as previously described[30].

Intracellular cytokine, degranulation and ^{51}Cr-release assays. Intracellular cytokine staining was performed as described[3]. To detect degranulation, splenocytes were stimulated for 5 h in the presence of brefeldin, monensin, anti-CD107a–FITC and anti-CD107b–FITC. ^{51}Cr-release assays were performed as described[3].

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1. Klenerman, P. & Hill, A. T cells and viral persistence: lessons from diverse infections. *Nature Immunol.* **6,** 873–879 (2005).
2. Wherry, E. J., Barber, D. L., Kaech, S. M., Blattman, J. N. & Ahmed, R. Antigen-independent memory CD8 T cells do not develop during chronic viral infection. *Proc. Natl Acad. Sci. USA* **101,** 16004–16009 (2004).
3. Wherry, E. J., Blattman, J. N., Murali-Krishna, K., van der Most, R. & Ahmed, R. Viral persistence alters CD8 T-cell immunodominance and tissue distribution and results in distinct stages of functional impairment. *J. Virol.* **77,** 4911–4927 (2003).
4. Zajac, A. J. *et al.* Viral immune evasion due to persistence of activated T cells without effector function. *J. Exp. Med.* **188,** 2205–2213 (1998).
5. Gallimore, A. *et al.* Induction and exhaustion of lymphocytic choriomeningitis virus-specific cytotoxic T lymphocytes visualized using soluble tetrameric major histocompatibility complex class I-peptide complexes. *J. Exp. Med.* **187,** 1383–1393 (1998).
6. Pantaleo, G. & Koup, R. A. Correlates of immune protection in HIV-1 infection: what we know, what we don't know, what we should know. *Nature Med.* **10,** 806–810 (2004).
7. Letvin, N. L. & Walker, B. D. Immunopathogenesis and immunotherapy in AIDS virus infections. *Nature Med.* **9,** 861–866 (2003).

8. Rehermann, B. & Nascimbeni, M. Immunology of hepatitis B virus and hepatitis C virus infection. *Nature Rev. Immunol.* **5,** 215–229 (2005).
9. Matloubian, M., Kolhekar, S. R., Somasundaram, T. & Ahmed, R. Molecular determinants of macrophage tropism and viral persistence: importance of single amino acid changes in the polymerase and glycoprotein of lymphocytic choriomeningitis virus. *J. Virol.* **67,** 7340–7349 (1993).
10. Ishida, Y., Agata, Y., Shibahara, K. & Honjo, T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J.* **11,** 3887–3895 (1992).
11. Nishimura, H., Nose, M., Hiai, H., Minato, N. & Honjo, T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity* **11,** 141–151 (1999).
12. Sharpe, A. H. & Freeman, G. J. The B7–CD28 superfamily. *Nature Rev. Immunol.* **2,** 116–126 (2002).
13. Chen, L. Co-inhibitory molecules of the B7–CD28 family in the control of T-cell immunity. *Nature Rev. Immunol.* **4,** 336–347 (2004).
14. Freeman, G. J. *et al.* Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J. Exp. Med.* **192,** 1027–1034 (2000).
15. Dong, H., Zhu, G., Tamada, K. & Chen, L. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. *Nature Med.* **5,** 1365–1369 (1999).
16. Matloubian, M., Concepcion, R. J. & Ahmed, R. $CD4^+$ T cells are required to sustain $CD8^+$ cytotoxic T-cell responses during chronic viral infection. *J. Virol.* **68,** 8056–8063 (1994).
17. Bevan, M. J. Helping the $CD8^+$ T-cell response. *Nature Rev. Immunol.* **4,** 595–602 (2004).
18. Shin, T. *et al. In vivo* costimulatory role of B7-DC in tuning T helper cell 1 and cytotoxic T lymphocyte responses. *J. Exp. Med.* **201,** 1531–1541 (2005).
19. Oflazoglu, E. *et al.* Paradoxical role of programmed death-1 ligand 2 in Th2 immune responses *in vitro* and in a mouse asthma model *in vivo*. *Eur. J. Immunol.* **34,** 3326–3336 (2004).
20. Egen, J. G., Kuhns, M. S. & Allison, J. P. CTLA-4: new insights into its biological function and use in tumour immunotherapy. *Nature Immunol.* **3,** 611–618 (2002).
21. Probst, H. C., McCoy, K., Okazaki, T., Honjo, T. & van den Broek, M. Resting dendritic cells induce peripheral $CD8^+$ T cell tolerance through PD-1 and CTLA-4. *Nature Immunol.* **6,** 280–286 (2005).
22. Tanchot, C. *et al.* Modifications of $CD8^+$ T cell function during *in vivo* memory or tolerance induction. *Immunity* **8,** 581–590 (1998).
23. Singh, N. J. & Schwartz, R. H. The strength of persistent antigenic stimulation modulates adaptive tolerance in peripheral $CD4^+$ T cells. *J. Exp. Med.* **198,** 1107–1117 (2003).
24. Iwai, Y., Terawaki, S., Ikegawa, M., Okazaki, T. & Honjo, T. PD-1 inhibits antiviral immunity at the effector phase in the liver. *J. Exp. Med.* **198,** 39–50 (2003).
25. Isogawa, M., Furuichi, Y. & Chisari, F. V. Oscillating $CD8^+$ T cell effector functions after antigen recognition in the liver. *Immunity* **23,** 53–63 (2005).
26. Dudley, M. E. & Rosenberg, S. A. Adoptive-cell-transfer therapy for the treatment of patients with cancer. *Nature Rev. Cancer* **3,** 666–675 (2003).
27. Autran, B., Carcelain, G., Combadiere, B. & Debre, P. Therapeutic vaccines for chronic infections. *Science* **305,** 205–208 (2004).
28. Wherry, E. J., Blattman, J. N. & Ahmed, R. Low CD8 T-cell proliferative potential and high viral load limit the effectiveness of therapeutic vaccination. *J. Virol.* **79,** 8960–8968 (2005).
29. Rodig, N. *et al.* Endothelial expression of PD-L1 and PD-L2 down-regulates $CD8^+$ T cell activation and cytolysis. *Eur. J. Immunol.* **33,** 3117–3126 (2003).
30. Kaech, S. M., Hemby, S., Kersh, E. & Ahmed, R. Molecular and functional profiling of memory CD8 T cell differentiation. *Cell* **111,** 837–851 (2002).

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