Cloning and sequencing *HAR1* and *NTS1*

A 72 kb region of BAC259.12D that encompasses the *HAR1* locus was sequenced in its entirety using a DNA Sequencing Kit (PE Applied Biosystems) with an automated DNA sequencer (ABI PRISM 3100; PE Applied Biosystems). *HAR1* complementary DNA was cloned from a cDNA library of *L. japonicus* shoots using DNA fragments of the first exon of the *HAR1* gene obtained by polymerase chain reaction (PCR) from BAC259-12D. After sequencing the cDNA clone, the full-length cDNA sequence was determined by 5′ rapid amplification of cloned ends (RACE) on *L. japonicus* shoot messenger RNA. The soybean gene showing the highest similarity with *HAR1*, *G. max CLV1B*, was amplified from the genomic DNA of a soybean cultivar Enrei and of a hypernodulating mutant, En6500, by PCR with a specific primer set designed from the DNA Data Bank of Japan (DDBJ) database. They were sequenced using a DNA Sequencing Kit (PE Applied Biosystems) with an automated DNA sequencer.

Complementation experiments

A *Sal*I–*Pvu*II fragment (12.5 kb) containing the entire *HAR1* gene was excised from a BAC clone, 259-12D, and ligated into the *Sal*I–*Sma*I site of binary vector pCAMBIA1301 (Cambia). It was transformed into *A. tumefaciens* AGL1 (provided by R. A. Ludwig) and used for transformation of *har1-4* and *har1-5*. Transgenic plants were produced by the hypocotyl transformation procedure[30], with hygromycin B as a selective marker. Seeds from transgenic lines were germinated in vermiculite pots, inoculated with *M. loti* strain TONO, and examined for plant growth and nodulation behaviour 24 days after inoculation.

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Correspondence and requests for materials should be addressed to M.K. (e-mail: masayosi@env.sc.niigata-u.ac.jp). The sequences for BAC259.12D-1, BAC259.12D-2, *L. japonicus HAR1* gene, and soybean *NTS1* gene have been deposited in the DDBJ database under accession numbers AB092808, AB092809, AB092810 and AB092811, respectively.

Self-recognition promotes the foreign antigen sensitivity of naive T lymphocytes

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Major histocompatibility complex (MHC) class I and II molecules are highly polymorphic proteins that bind and present foreign peptides to the clonally distributed αβ receptors (TCR) of T lymphocytes. As a population, the immature T lymphocytes generated in the thymus express a very diverse set of TCR specificities. A process of positive selection filters this broad repertoire to optimize peripheral T cells for antigen recognition in the context of available MHC products. Only those precursor T cells whose TCRs generate an adequate but not excessive signalling response to self-peptides bound to the expressed MHC proteins undergo successful maturation[1]. Here we show that post-thymic self-recognition facilitates the antigen reactivity of mature T cells. Both experimental and physiological interruption of T-cell contact with self-peptide MHC ligands leads to a rapid decline in signalling and response sensitivity to foreign stimuli. Because the adaptive immune system must be recruited early in an infectious process when antigen is limiting[2], these findings suggest that positive selection ensures predictable T-cell recognition of available self-ligands, which in turn promotes efficient responses to pathogens.

One of the consequences of positive selection is the maintenance of the TCRs of naive T cells in a state of partial phosphorylation,

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owing to recognition of self-peptide MHC molecule ligands in peripheral lymphoid tissues[3,4]. This continuous subthreshold signalling might enhance peripheral T-cell sensitivity to a low level of foreign antigen in a manner analogous to how weak signals facilitate response in other biological systems[5]. Alternatively, because a similar partial phosphorylation pattern is induced by inhibitory ligands of the TCR (antagonists), self-recognition by mature T cells could have an inhibitory influence that might control autoimmunity[6]. To distinguish between these divergent hypotheses, we examined the signalling and activation properties of naive T cells deprived of self-MHC contact. Removing TCR transgenic $CD4^+$ T cells from secondary lymphoid tissues and placing them in dispersed culture at 37 °C for as little as 15–30 min led to a rapid loss of pre-existing partial TCR ζ-chain phosphorylation (Fig. 1a) and substantial blunting of proliferation in response to foreign antigen compared with cells that were stimulated immediately after isolation (Fig. 1b). Control studies demonstrated that the depressive effects of pre-culture were not explained by a loss of viability among the T cells or by pre-sensitization to TCR-signalled death (Supplementary Fig. 1). This rapid loss of sensitivity in culture may explain the more extensive *in vivo* activation of p38 mitogen-activated protein kinase in TCR transgenic T cells by specific antigen, as compared with T cells stimulated *in vitro* after an isolation that included an exposure of cells to 37 °C for 25 min[7].

To examine the consequences of *in vivo* loss of self-recognition, TCR transgenic animals ($H–2^b$) were injected with monoclonal antibody to the MHC class II molecule involved in positive selection. Cells recovered 36 h after such an injection were stimulated with foreign antigen. As with the cells placed in dispersed culture at 37 °C, the T cells deprived of self-recognition *in vivo* showed a substantial loss in TCR signalling sensitivity and functional antigen responsiveness (proliferation) in comparison with those recovered from mock-treated mice (Fig. 2a–d). Similar effects were seen as early as 8 h after antibody injection (data not shown). Signalling for an early activation response with a very low signalling threshold (CD69 upregulation[8]) was comparable for control cells and cells from animals treated with antibody (Fig. 2e). Furthermore, all self-ligand-deprived T cells underwent a cell size increase in response to agonist ligand that was comparable after 16 h to that of control cells (Supplementary Fig. 2b, c). Taken together, these results indicate that the MHC-deprived T cells retained some capacity for receptor signalling. The loss of antigen sensitivity induced by interference with self-recognition became detectable by cell size analysis after 31 h of incubation (Supplementary Fig. 2b, c), and was evident when the T cells were analysed for synthesis of the cytokine interleukin-2 (IL-2), a response that requires a higher intensity and more prolonged TCR signal[9] (Fig. 2f). No evidence of enhanced spontaneous or TCR-induced death was seen among the cells from animals treated with antibody (Fig. 2g).

The preceding experiments did not address the role of MHC polymorphism in sustaining T-cell sensitivity. In addition, binding an antibody to MHC class II molecules on antigen-presenting cells (APCs) *in vivo* can potentially alter the physiology of the presenting cell itself or act by blocking signalling through the MHC class II-binding CD4 co-receptor[10]. Therefore, $CD4^+$ TCR transgenic cells were transferred to recipients that either lacked detectable MHC class II molecule expression[11] or that expressed an MHC class II allele ($H–2^q$) distinct from that involved in the positive selection. The former tested the general role of MHC class II molecules in maintaining lymphocyte sensitivity, whereas the latter specifically examined the role of TCR self-recognition in distinction to the MHC class II–CD4 interaction. In both cases, the recovered T cells showed a similar loss of foreign antigen sensitivity relative to cells transferred into and recovered from an environment expressing the selecting MHC class II molecule (Fig. 2h). No signs of alloreactive responses of $H–2^q$ $CD8^+$ T cells were observed in those cultures lacking pigeon cytochrome *c* (PCC) peptide or in control co-cultures of fresh AND T cells and $H–2^q$ lymph node cells (Supplementary Fig. 3).

To determine whether these findings apply to a polyclonal population of T cells, β2 microglobulin-deficient C57BL/6 mice with a normal population of $CD4^+$ T cells but lacking most $CD8^+$ T cells[12] were injected with blocking monoclonal antibody against MHC class II molecules. Recovered lymphocytes were then exposed to the bacterial superantigen staphylococcal enterotoxin A (SEA), which stimulates T cells expressing diverse TCRs[13]. The polyclonal $CD4^+$ T cells from animals in which MHC class II recognition was blocked showed a marked loss of sensitivity to TCR stimulation as compared with cells from control mice (Fig. 2i, j). Similar results were obtained with staphylococcal enterotoxin B (SEB) (data not shown).

To place these observations in a physiological context, we examined an *in vivo* circumstance in which $CD4^+$ T cells are temporarily without the opportunity for self-MHC molecule recognition; that is, during their transit between secondary lymphoid tissues by means of the blood[14]. Freshly isolated, blood-derived TCR transgenic T cells had nearly undetectable levels of partially phosphorylated TCR ζ and displayed lower sensitivity to foreign antigen compared with T cells from secondary lymphoid tissue (Fig. 3). This is the same biochemical and functional pattern seen with cells experimentally deprived of TCR interaction with self-peptide MHC ligands (Figs 1 and 2).

Several factors may contribute to the self-induced maintenance of antigen sensitivity. Subthreshold stimulation may promote cellular responses through increases in the local concentration of signalling molecules[5], such as the modification/recruitment of various pro-activating components of the TCR signal transduction apparatus, including binding of the kinase ZAP-70 to partially phosphorylated TCR ζ chains. Furthermore, recognition of self-ligand may promote a particular spatial organization of TCRs that could facilitate signalling in response to stronger ligands, in analogy to the clustering of glutamate receptors induced by low-level spontaneous neurotransmitter release at neuromuscular junctions[15]. This clustering is essential for fast and reliable transmission of action potentials. We therefore examined the distribution of TCR complexes on T cells *in situ*. Immunofluorescent staining of lymph node sections revealed a crescent-shaped polarization of TCRs to the region of the lymphocyte membrane that was apposed to the MHC class II-rich membrane of interdigitating cells with a dendritic morphology (Fig. 4a). An asymmetric distribution of TCRs was also seen on most of the $CD4^+$ T cells freshly isolated from lymph

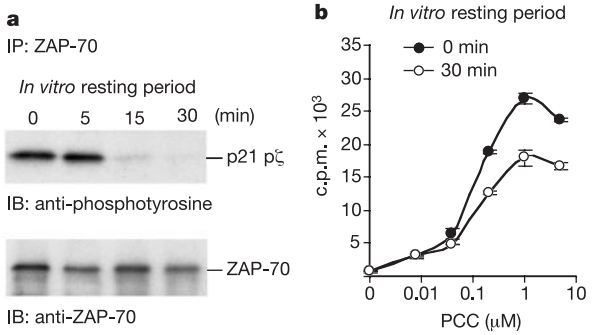

Figure 1 Acute loss of TCR ζ phosphorylation and antigen sensitivity by cultured naive T cells. **a**, **b**, Naive 5C.C7 $CD4^+$ T cells were isolated from lymph nodes in ice-cold medium and either stimulated immediately or warmed to 37 °C for varying times. The cells were lysed and the proteins subjected to immunoprecipitation (IP) and immunoblot (IB) analysis for tyrosine phosphorylation of the TCR ζ chain (**a**). Alternatively, freshly isolated or cultured cells were mixed with DCEK-presenting cells and varying concentrations of PCC peptide 88–104, then assayed at 60 h for proliferation using ^{3}H-thymidine incorporation (**b**).

nodes (Fig. 4b–d). In agreement with our observations of self-ligand-sustained TCR ζ phosphorylation and signalling sensitivity, TCR polarization diminished within 30 min of *in vitro* culture at 37 °C (Fig. 4b–d), after injection of anti-MHC class II antibody *in vivo* (Fig. 4e, f), or following physiological loss of contact with MHC class II molecules by $CD4^+$ T cells in the bloodstream (Fig. 4g).

These findings support the hypothesis that self-recognition maintains the sensitivity of T cells. They stand in contrast to predictions of the depression of T-cell responsiveness by self-ligand engagement that were based on an analogy to antagonist ligand function. To better understand why T cells behave differently in these two situations (self-recognition compared with antagonist exposure) despite similar patterns of TCR subunit phosphorylation,

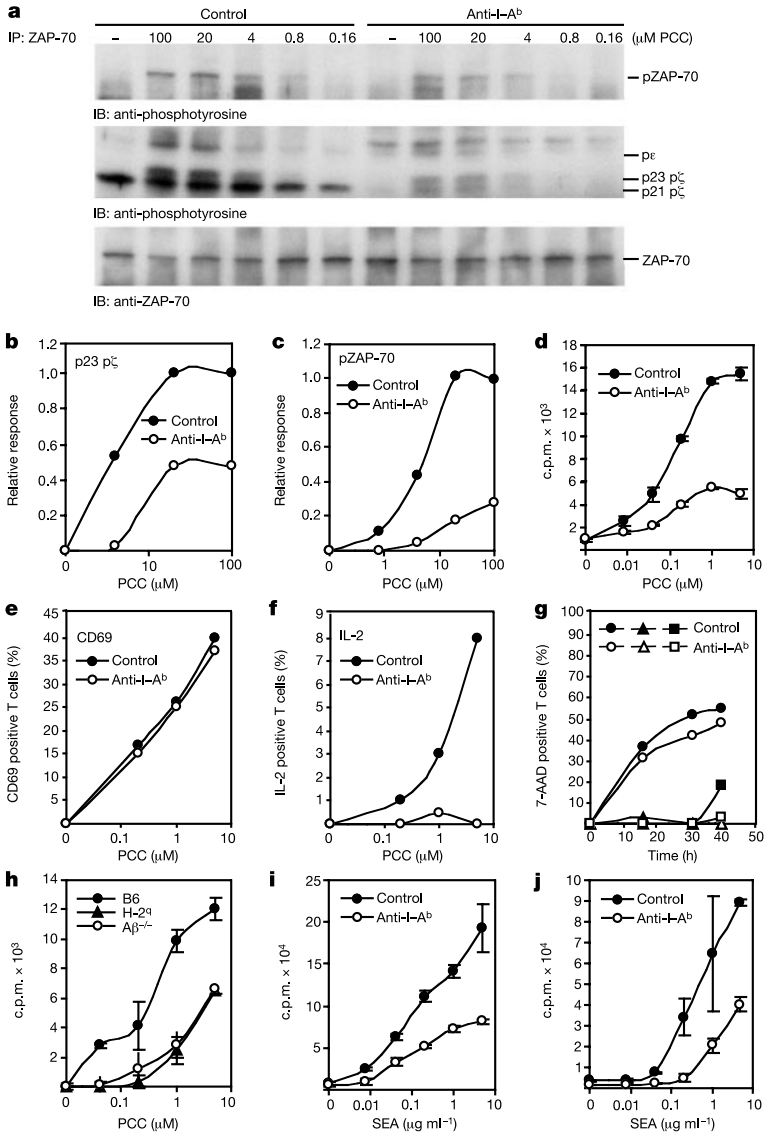

Figure 2 Marked decline in TCR signalling and sensitivity to foreign antigen among naive $CD4^+$ T cells deprived of self-MHC class II recognition *in vivo*. **a**–**d**, AND H–2^b mice were either mock-injected or injected with monoclonal antibody Y3P specific for the peptide-binding domain of I–A^b—the only MHC class II molecule expressed in these animals and responsible for positive selection of the naive AND $CD4^+$ T cells. **a**, Phosphotyrosine immunoblot analysis of the TCR proximal signalling response of freshly isolated cells from control and anti-I–A^b-treated animals across a range of offered PCC peptide using DCEK as presenting cells. These data are quantified in **b** (p23 phospho-ζ) and **c** (phospho-ZAP-70). Relative response was calculated as a ratio between the density for each experimental condition and the density of p23 phospho-ζ or phospho-ZAP-70 in control T cells exposed to APCs pulsed with 100 μM PCC. **d**, Proliferative responses of lymph node cells from control and anti-MHC class II antibody-treated animals stimulated with PCC peptide. **e**–**g**, Single cell analyses of T cells isolated from mock-injected and anti-I–A^b antibody (Y3P) injected AND H–2^b mice. T cells were placed in culture with DCEK APCs pre-pulsed with the indicated concentrations of PCC peptide, and analysed after 6 h for cell surface expression of CD69 (**e**) and intracellular IL-2 (**f**). **g**, Cell viability was assayed over a 40-h period using 7-aminoactinomycin D. Circles, viability values of T cells incubated without added APCs; triangles, T cells cultured in the presence of APCs; squares, T cells stimulated in the presence of APCs pre-pulsed with 5 μM PCC. **h**, Naive $CD4^+$ T cells from H–2^b AND donors were labelled with CFSE and transferred intravenously into wild-type H–2^b, *Aβ* $^{-/-}$, or wild-type H–2^q recipients. Thirty-six hours later, lymph nodes were recovered and equal numbers of unpurified transferred cells were placed in culture with I–E^k-expressing DCEK cells and various concentrations of PCC peptide. ^{3}H-thymidine incorporation as an indicator of cell proliferation was measured 80 h later. **i**, **j**, *β2m* $^{-/-}$ H–2^b mice were either mock-injected or injected with Y3P antibody to I–A^b to block MHC class II recognition. Thirty hours later, lymph node cells were placed in culture with either DCEK (**i**) or B10.A splenic presenting cells (**j**) and the indicated concentration of SEA. ^{3}H-thymidine incorporation was measured 60 h later as an indication of the proliferative response.

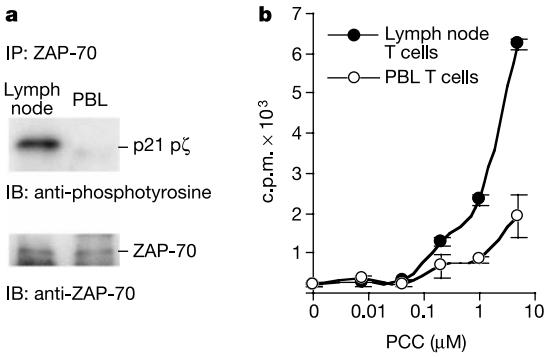

Figure 3 Peripheral blood CD4$^+$ T cells lacking MHC class II contact have lower TCR ζ phosphorylation and reduced antigen sensitivity compared with lymph node cells from the same animals. **a**, **b**, Peripheral blood and lymph node CD4$^+$ T cells from H–2^b AND mice were isolated and analysed by immunoprecipitation and phosphotyrosine immunoblotting for the state of the TCR ζ-chain phosphorylation (**a**), or placed in culture with B10.A splenic presenting cells and stimulated with PCC peptide for 60 h before analysis of proliferation using ^{3}H-thymidine incorporation (**b**). Longer exposure of the blot in **a** revealed a low level of phosphorylated ζ-chains in anti-ZAP-70 immunoprecipitates from PBLs (data not shown).

we examined the distribution of the inhibitory tyrosine phosphatase SHP-1. This enzyme is recruited to TCRs engaging antagonist ligands[16], where it interferes with proximal signalling. T cells exposed to antagonist-bearing APCs showed extensive membrane association of SHP-1 in the region of receptor polarization, whereas freshly isolated CD4$^+$ T cells showed relatively little SHP-1 accumulation in the region of clustered TCRs (Fig. 4h, i). These results are concordant with the functional data reported above, as well as with evidence for differences between freshly isolated and antagonist-stimulated T cells in the specific sites of ζ-chain tyrosine phosphorylation[17].

Positive selection of T cells in the thymus is a phenomenon recognized for nearly 25 years. Here we provide evidence for a previously unappreciated consequence of this process. Positive selection ensures that peripheral T cells express TCRs capable of functional binding to the pool of self-ligands available before introduction of a foreign ligand. In turn, this predictable TCR engagement actively sustains the naive T cells in an optimal state of sensitivity; that is, they are prepared to respond to a low density of foreign peptide MHC molecule ligand early in an infection[2]. The rapid loss of this sensitive state on disengagement from self-MHC ligands suggests that it can be considered as a form of 'short-term memory'. Both biochemical modification of the TCR signalling

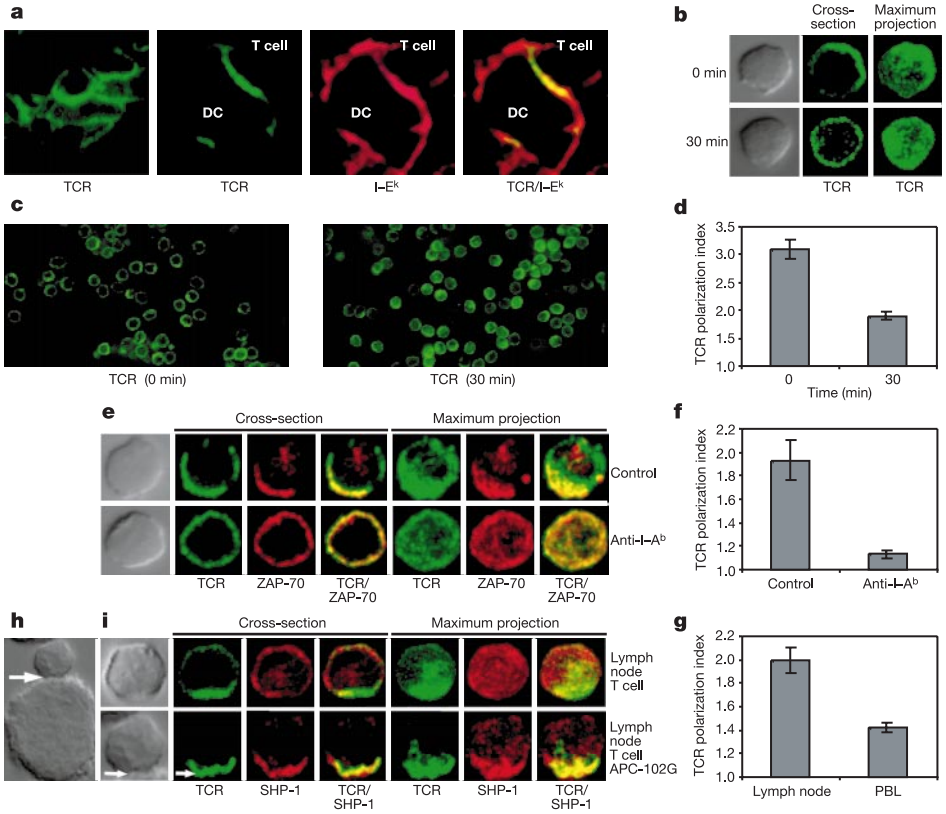

Figure 4 Naive CD4$^+$ T cells show polarized TCR distributions that are maintained by contact with self-MHC class II molecules. **a**, Frozen sections of 5C.C7 lymph nodes were stained for TCR β-chain (green) and I–E^k (red). The left panels show the TCR staining of a cluster of T cells. The right panels show the TCR and MHC class II staining of a single T cell–presenting cell pair. Co-localized TCR and MHC class II appear yellow in the superimposed image. DC, dendritic cells. **b**–**d**, 5C.C7 CD4$^+$ T cells were either fixed immediately after isolation or after incubation for 30 min at 37 °C and stained with anti-TCR β (**b**). Whole-field views of freshly isolated and *in vitro* rested T cells are shown in **c**. The frequency and degree of TCR polarization, expressed as the TCR polarization index (see Methods), is shown in **d**. **e**, **f**, Freshly isolated CD4$^+$ T cells from control and anti-I–A^b-treated AND mice were fixed, stained for either TCR β (green) or ZAP-70 (red) (**e**), and the TCR polarization index was calculated (**f**). **g**, CD4$^+$ T cells from AND mice isolated from lymph nodes or blood were fixed, stained for TCR β, and the TCR polarization index was calculated. **h**, **i**, Naive CD4$^+$ T cells from lymph nodes of 5C.C7 transgenic mice were either fixed immediately or stimulated for 1 min with DCEK presenting cells pre-pulsed with 100 μM antagonist peptide MCC (102G) and fixed. A full interference contrast view of the T cell–APC conjugate is shown in **h**. The cells were first stained for TCR (green), then permeabilized and stained for SHP-1 (red) (**i**). Arrows indicate contact between T cell and APC.

environment and the self-ligand-sustained polarization of the TCRs we report here may contribute to this effect. Concentration of TCRs increases the likelihood that contact with an APC bearing low densities of foreign ligand will result in a sufficient number of engagement events to trigger cell activation. Greater TCR density would also increase the effective on-rate of the binding reaction. Finally, receptor clustering can facilitate signal transduction, possibly by promoting biochemical crosstalk among nearby receptors[18–20]. Induction of elevated levels of the inhibitory surface molecule CD5 (ref. 21) does not seem to override the positive effects of self-recognition. Peripheral blood $CD4^+$ T cells show slightly lower CD5 levels than lymph node T cells from the same animals (data not shown), but are less sensitive despite this. In one report, $CD4^+$ T cells deprived of self recognition *in vivo* did not show a difference in response compared to control cells[3]. However, the comparison involved cells subjected to a period of *in vitro* manipulation before stimulation, which may have led to a loss of sensitivity among those from the self-ligand-containing environment.

Evidence has emerged that MHC molecule binding can sustain memory $CD4^+$ T cells in a state of heightened antigen reactivity[22]. Other studies have shown that co-presentation of 'null' ligands can augment T-cell responses at low densities of foreign ligand[23,24], raising the possibility that self-recognition may also contribute to T-cell activation concurrent with foreign antigen recognition. These data are in accord with the conclusion reached here, that self-recognition has a role in augmenting immunity rather than constraining it. □

Methods

Mice

All TCR transgenic mice were *Rag2*$^{-/-}$ mice[25] that were homozygous for the TCR transgene. C57BL/6NCr Ly5.1 congenic mice (B6 H–2^b) were obtained from the National Cancer Institute. B6 $\beta 2m^{-/-}$ (ref. 12) and B10.D1(H–2^q) mice were purchased from Jackson Laboratories. B6 $A\beta^{-/-}$ mice[11], H–2^b AND TCR transgenic[26] *Rag2*$^{-/-}$ mice, and 5C.C7 TCR transgenic[27] *Rag2*$^{-/-}$ mice were obtained from the National Institute of Allergy and Infectious Diseases contract colony maintained at Taconic Farms.

In vitro resting and antibody injections

For *in vitro* resting, cells were incubated at 37 °C for the indicated time in RPMI-1640 with 10% heat-inactivated fetal calf serum. For *in vivo* MHC class II blocking, mice were injected intraperitoneally with 1 to 2 mg of anti-I–A^b (clone Y3P[28]) in two or three separate intraperitoneal injections over 30–48 h preceding recovery of lymph node cells. When TCR transgenic mice were used, splenocytes were recovered and combined with the lymph node cells. Use of anti-I–A^b in AND TCR transgenic mice prevents the antibody treatment from interfering with subsequent I–E^k-dependent functional assays.

Peripheral blood cell analysis

Peripheral blood lymphocytes (PBLs) were obtained from a retro-orbital bleed performed under anaesthesia in accordance with NIH veterinary standards. Blood was collected into heparinized Monoject tubes (Sherwood Medical). Erythrocytes were lysed using ACK lysing buffer (Quality Biological). MHC class II-expressing cells were removed from both PBLs and T cells collected from lymph nodes as described below to eliminate their potential contributions in functional experiments. To verify that the processing of PBL T cells does not influence their sensitivity status, T cells removed from lymph nodes were added into blood collected from T-cell-deficient donors (*CD3ε*$^{-/-}$ B10.A), treated identically to PBL T cells, and their responsiveness compared to T cells freshly isolated from lymph nodes. Proliferative responses were comparable for both groups.

AND transgenic T-cell transfers

$CD4^+$ T cells were enriched from a mixture of H–2^b AND TCR transgenic lymph node and spleen cells by negative selection at 4 °C using M450 sheep anti-rat immunoglobulin-γ (IgG) magnetic beads (Dynal Corp.) following incubation of the cells with a cocktail of antibodies that included anti-I–A^b (B21-2), anti-B220 (RA3-3A1), anti CD11c (N418), anti-CD11b (M1/70), anti-DEC-205 (NLDC-145) and anti-FcγRII/III (2.4G2). Hybridoma cell culture supernatants were used except for purified M1/70, which was a gift from B. Kelsall and A. Iwasaki. Enriched $CD4^+$ T cells were dye-labelled for 5 min with 0.3 μM 5-(and 6-) carboxyfluorescein diacetate succinamidyl ester (CFDA-SE or CFSE; Molecular Probes), washed, and injected intrevenously into the indicated recipients. Recipients were pretreated with 300–500 μg purified anti-NK1.1 (PK136). Twenty-four to thirty-six hours after transfer, lymph node cells were recovered from the recipients. Recovered lymph node cells containing comparable numbers of transferred, labelled AND T cells per assay well were stimulated *in vitro* as described below without further purification.

Antigen/superantigen stimulation and proliferation

Stimulator cells were I–E^k-expressing, B7.1$^{negative\text{-}low}$, ICAM-1^{negative} fibroblasts (DCEK[29]) or I–E^k-expressing B10.A splenocytes, exposed to 3,000 rad γ-irradiation before use. Comparable results were obtained using either type of presenting cell. The stimulator cells were pre-incubated for 4 h with the indicated concentration of peptide (PCC 88–104) or staphylococcal enterotoxin A (SEA, Toxin Technologies). For B6 $\beta 2m^{-/-}$ responders, lymph node cells were used unpurified as responders. T cells and washed stimulator cells were incubated together for 48–72 h (TCR transgenic T cells, 5×10^4 per well except for cell transfers) or 72–120 h (polyclonal T cells, 1×10^5 per well and cell transfer experiments, $0.5–1 \times 10^4$ AND T cells per well) in 96-well plates. A total of 1 or 2 μCi ^{3}H-thymidine (New England Nuclear) was added to each well, and cultures were incubated for a further 8–15 h before incorporation was measured.

Single cell response analysis

The T cells were stimulated with DCEK cells pre-pulsed with PCC peptide and analysed after 6 h for CD69 and IL-2 expression[30], and at the indicated time points for viability using 1-aminoactinomycin D staining (Sigma).

Immunoprecipitation and immunoblot analysis

For signalling analysis, DCEK stimulator cells were used and cells were stimulated for 1 min before pelleting and lysis. Cells were lysed in buffer containing 1% NP–40 (Piece) with protease and phosphatase inhibitors. ZAP-70 precipitation and protein or phosphotyrosine immunoblotting were performed as described[16].

Immunofluorescence microscopy

For immunohistochemistry, 17-μm frozen sections of lymph node were used. For immunofluorescence staining, freshly isolated T cells were first fixed in 4% paraformaldehyde in PBS for 5 min at 37 °C. Antibodies, fluorochromes, staining procedures, and methods for confocal analysis and quantification are provided in the Supplementary Information.

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HIV-1 superinfection despite broad CD8$^+$ T-cell responses containing replication of the primary virus

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Early treatment of acute HIV-1 infection followed by treatment interruptions has shown promise for enhancing immune control of infection[1–3]. A subsequent loss of control, however, allows the correlates of protective immunity to be assessed. Here we show that sudden breakthrough of plasma viraemia occurred after prolonged immune containment in an individual infected with HIV-1 at a time when 25 distinct CD8$^+$ T-cell epitopes in the viral proteins Gag, RT, Integrase, Env, Nef, Vpr, Vif and Rev were being targeted. Sequencing of the virus in plasma and cells showed that superinfection with a second clade-B virus was coincident with the loss of immune control. This sudden increase in viraemia was associated with a decline in half of the CD8$^+$ T-cell responses. The declining CD8$^+$ T-cell responses were coupled with sequence changes relative to the initial virus that resulted in impaired recognition. Our data show that HIV-1 superinfection can occur in the setting of a strong and broadly directed virus-specific CD8$^+$ T-cell response. The lack of cross-protective immunity for closely related HIV-1 strains, despite persistent recognition of multiple CD8 epitopes, has important implications for public health and vaccine development.

Increasing evidence suggests that virus-specific CD8$^+$ T-cell responses are important in the control of HIV-1 or SIV replication[4–8], and inducing and maintaining these responses are considered to be central for the development of effective HIV-1 vaccines. In the simian-HIV (SHIV) model, induction of strong SHIV-specific CD8$^+$ T-cell responses does not prevent infection after challenge but is associated with persistent control of low levels of viral replication and attenuated disease[9–11]. Although similar vaccine regimens have failed to control more pathogenic and less easily neutralizable SIV strains[12–14], these studies nevertheless provide optimism that an effective vaccine to prevent HIV-1 disease progression in humans could be developed. Indeed, the augmentation of HIV-1-specific immunity by the early treatment of persons with acute HIV-1 infection, followed by supervised treatment interruptions (STI), has been shown[1–3]. Most individuals treated by this method have been able to control, at least transiently, viraemia after the cessation of treatment at levels that did not require the re-initiation of treatment[3]. One such individual effectively controlled infection for over 290 d after STI but then experienced a marked increase in plasma viral load (Fig. 1a), offering us the opportunity to investigate the immunological and virological

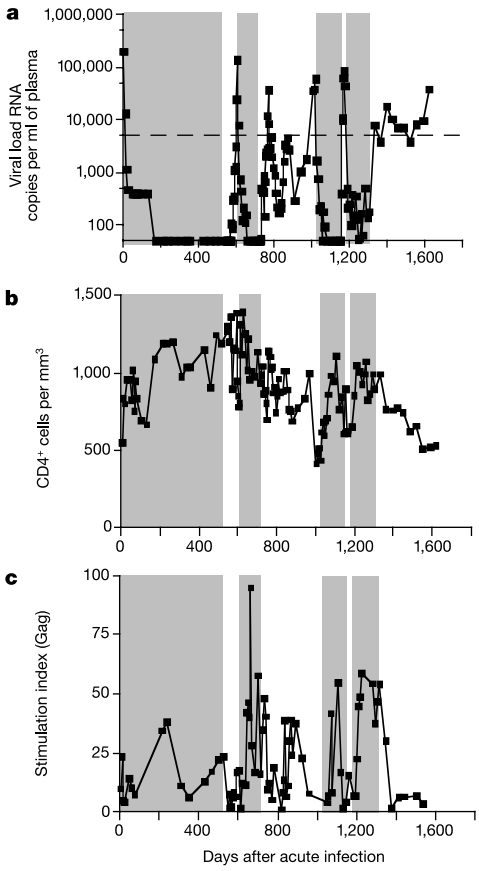

Figure 1 HIV-1 viral loads, CD4$^+$ T-cell counts and Gag-specific lymphoproliferative responses in study subject AC-06. **a**, HIV-1 plasma viral loads, **b**, CD4$^+$ T-cell counts; **c**, Gag-specific lymphoproliferative responses. Time zero represents the first presentation of AC-06 with symptomatic acute HIV-1 infection. At this time, HIV-1 viral loads were 8.8×10^6 RNA copies per ml of plasma (not shown). Shaded areas represent treatment with highly active antiretroviral therapy (HAART). Dotted line indicates 5,000 copies of HIV-1 RNA per ml of plasma.