The immune response during acute HIV-1 infection: clues for vaccine development

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Abstract | The early immune response to HIV-1 infection is likely to be an important factor in determining the clinical course of disease. Recent data indicate that the HIV-1 quasispecies that arise following a mucosal infection are usually derived from a single transmitted virus. Moreover, the finding that the first effective immune responses drive the selection of virus escape mutations provides insight into the earliest immune responses against the transmitted virus and their contributions to the control of acute viraemia. Strong innate and adaptive immune responses occur subsequently but they are too late to eliminate the infection. In this Review, we discuss recent studies on the kinetics and quality of early immune responses to HIV-1 and their implications for developing a successful preventive HIV-1 vaccine.

Recent advances that enable the identification of patients within the first few weeks of HIV-1 infection[1,2] have provided researchers access to samples from acutely infected patients earlier and in higher numbers than previously available. This has advanced our understanding of the nature of the transmitted virus and the first immune responses in the period before establishment of stable viraemia (the viral set point), which occurs 3–6 months after infection. The first weeks following HIV-1 transmission are extremely dynamic: they are associated with rapid damage to generative immune cell microenvironments, caused by direct viral cytopathicity and bystander effects, and with immune responses that partially control the virus.

In this Review, we focus our discussion on the early host or viral factors that are crucial for determining the outcome of HIV-1 infection. These include the nature of the transmitted virus, or founder virus, suppression of the initial infection by genetically influenced immune responses, and the rate of virus mutation and viral fitness of selected mutants. In addition, we review what is known about the nature of innate and adaptive immune responses during this early phase of infection, drawn from studies of humans and macaques infected with HIV-1 and simian immunodeficiency virus (SIV), respectively. Finally, we discuss how our knowledge of the events of early HIV-1 infection can improve the design of a preventive vaccine (BOX 1).

The biology of early HIV-1 infection

Transmission. Most HIV-1 infections occur by sexual exposure through the genital tract or rectal mucosa. Although it is not possible to study the very first events following HIV-1 transmission in humans *in vivo*, we have gained some understanding from studies in which mucosal tissue explants were infected *in vitro*[3–5]. Further understanding of the first stages of infection *in vivo* has been obtained from studies in which macaques were inoculated intrarectally or intravaginally with SIV[6,7]. It is still uncertain whether HIV-1 is transmitted as a free or a cell-bound virus, but SIV can be transmitted in either form[8]. In addition, the mechanism by which HIV-1 crosses the genital mucosal epithelium is unclear. Diffusion of HIV-1 across the vaginal mucosa is slowed by cervico-vaginal mucus[9]. It is possible that virus that reaches the mucosal epithelium crosses this barrier by transcytosis or by making direct contact with dendrites of intraepithelial dendritic cells (DCs). Preliminary unpublished findings suggest that virions may also move through intercellular spaces in the epithelium to make initial cell contact with underlying mucosal Langerhans cells and CD4+ T cells (T. Hope and S. McCoombe, personal communication). Given that multiple sexual exposures are usually needed for infection to occur, crossing of the epithelial cell barrier by the virus is probably a rare event, although it is more common if the genital mucosa is damaged by physical trauma or co-existing genital infections[10,11].

Box 1 | **Problems facing the development of an HIV vaccine**

All attempts to make a vaccine against HIV-1 have failed. Three vaccine approaches have been tested in clinical trials for efficacy. The AIDSVAX glycoprotein (gp)120 vaccine stimulated the production of non-neutralizing antibody to the virus envelope proteins and failed to protect vaccinated individuals from infection[153]. The STEP vaccine, comprised of three recombinant attenuated adenovirus serotype 5 viruses expressing HIV-1 Gag, Pol and Nef, stimulated CD8+ T cell responses to the viral proteins but again showed no protective effect[154,155]. Similar virus vector-based vaccines have been shown to stimulate simian immunodeficiency virus (SIV)-specific CD8+ T cell responses in rhesus macaques, and an adenovirus serotype 5 vector expressing Gag protected against challenge with a chimeric SIV–HIV (SHIV89.6p) virus but was not protective against challenge with the more natural SIVmac239 (REF. 156). More recent data show that recombinant vaccines that stimulate much broader and stronger CD8+ T cell responses can partially protect against SIVmac239 and SIVmac251 virus challenge, resulting in more attenuated infection with low virus load and prolonged survival of rhesus macaques[113,157].

A third efficacy trial, in Thailand using a canary pox viral vector expressing gp120, Gag and Pol to prime immune responses followed by the AIDSVAX gp120 vaccine to boost the immune response, has been reported recently[146]. This showed for the first time a small protective effect, with 30% fewer vaccine recipients becoming infected with HIV-1 than controls; the result was statistically significant in one of the three analyses made. The volunteer cohort was low risk (annual incidence of infection ~0.3%) and this may be relevant as it may be easier to protect such people than those at high risk. It is not clear whether protection was mediated by antibody, T cells, innate cells or some combination of the three, but those who did become infected did not have reduced virus levels, which is usually seen for protection mediated by T cells in SIV models[113,157].

There is a general consensus in the field that future vaccine approaches should be less empirical and that a deeper understanding of the earliest immune responses to HIV-1 and SIV infection is needed. It will also be important to understand why broad-specificity, neutralizing antibodies are not routinely induced and to determine ways to safely induce them, and to identify what immune responses lead to a better outcome — as in the rare individuals, known as 'elite controllers', who successfully control HIV-1 infection for decades without needing antiretroviral drug therapy[158,159].

Eclipse phase. Following transmission of the virus, there is a period of ~10 days, known as the eclipse phase, before viral RNA becomes detectable in the plasma (FIG. 1). Single-genome amplification and sequencing of the first detectable virus has shown that ~80% of mucosally transmitted HIV-1 clade B and C infections are initiated by a single virus[12–14]. Infectious molecular clones derived from these primary founder viruses could infect CD4+ T cells with greater efficiency than they could infect monocytes and macrophages[14], which differs from the virus quasispecies that arise later in the infection and can infect lymphoid and myeloid cell types with equal efficiency. Studies in rhesus macaques inoculated intra-rectally with a complex SIV quasispecies also showed that productive infection arises from a single infecting virus[15], which supports the use of SIV infection of rhesus macaques as a model for HIV-1 transmission and vaccine studies. In other studies[7,16] in which macaques were infected experimentally, the first cells to be infected in the vaginal mucosa were found in foci of resident memory T cells that expressed the virus receptors CD4 and CC-chemokine receptor 5 (CCR5), which is consistent with the cell tropism of cloned HIV-1 founder virus[14].

Homogeneity of the founder virus indicates that the established infection probably arises from a single focus of infected mucosal CD4+ T cells. Virus replication at this focus might in fact be supported by early innate immune responses that lead to the recruitment of additional susceptible T cells to the site[16]. The failure of most infected foci to become established may be explained by the high error rate in reverse transcription that occurs during HIV-1 replication and the effects of the host antiviral apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like (APOBEC) cytidine deaminases APOBEC3G and APOBEC3F, which cause many viruses produced in infected CD4+ T cells to be defective[17].

Peak viraemia. At the end of the eclipse phase, virus and/or virus-infected cells reach the draining lymph node, where they meet activated CD4+CCR5+ T cells, which are targets for further infection. This process is augmented by DCs that bind and internalize virus through DC-specific ICAM3-grabbing non-integrin (DC-SIGN; also known as CD209) and carry the virus to activated T cells[18]. B cells may also be involved in the early spread of infection by binding the virus through the complement receptor CD21 (also known as CR2)[19]. The virus then replicates rapidly and spreads throughout the body to other lymphoid tissues, particularly gut-associated lymphoid tissue (GALT), where activated CD4+CCR5+ memory T cells are present in high numbers[20–22]. Approximately 20% of CD4+ T cells in the GALT are infected in both humans with acute HIV-1 infection and SIV-infected macaques. Up to 60% of uninfected CD4+ T cells at this site become activated and die by apoptosis, resulting in the release of apoptotic microparticles that can suppress immune function[23]. Therefore, ~80% of CD4+ T cells in the GALT can be depleted in the first 3 weeks of HIV-1 infection[20,24,25]. While HIV-1 is replicating in the GALT and other lymphoid tissues, the plasma viraemia increases exponentially to reach a peak, usually more than a million RNA copies per ml of blood, at 14–21 days after SIV infection in macaques and at 21–28 days after HIV-1 infection in humans (FIG. 1). CD4+ T cell numbers are low at the time of peak viraemia but later return to near normal levels in the blood but not in the GALT[20,24,25]. Although B cells are not depleted during early HIV-1 infection, B cell responses are impaired owing to the destruction of other cell types that are important for the development of germinal centres. Up to 50% of germinal centres in the gut are lost within the first 80 days of infection[26].
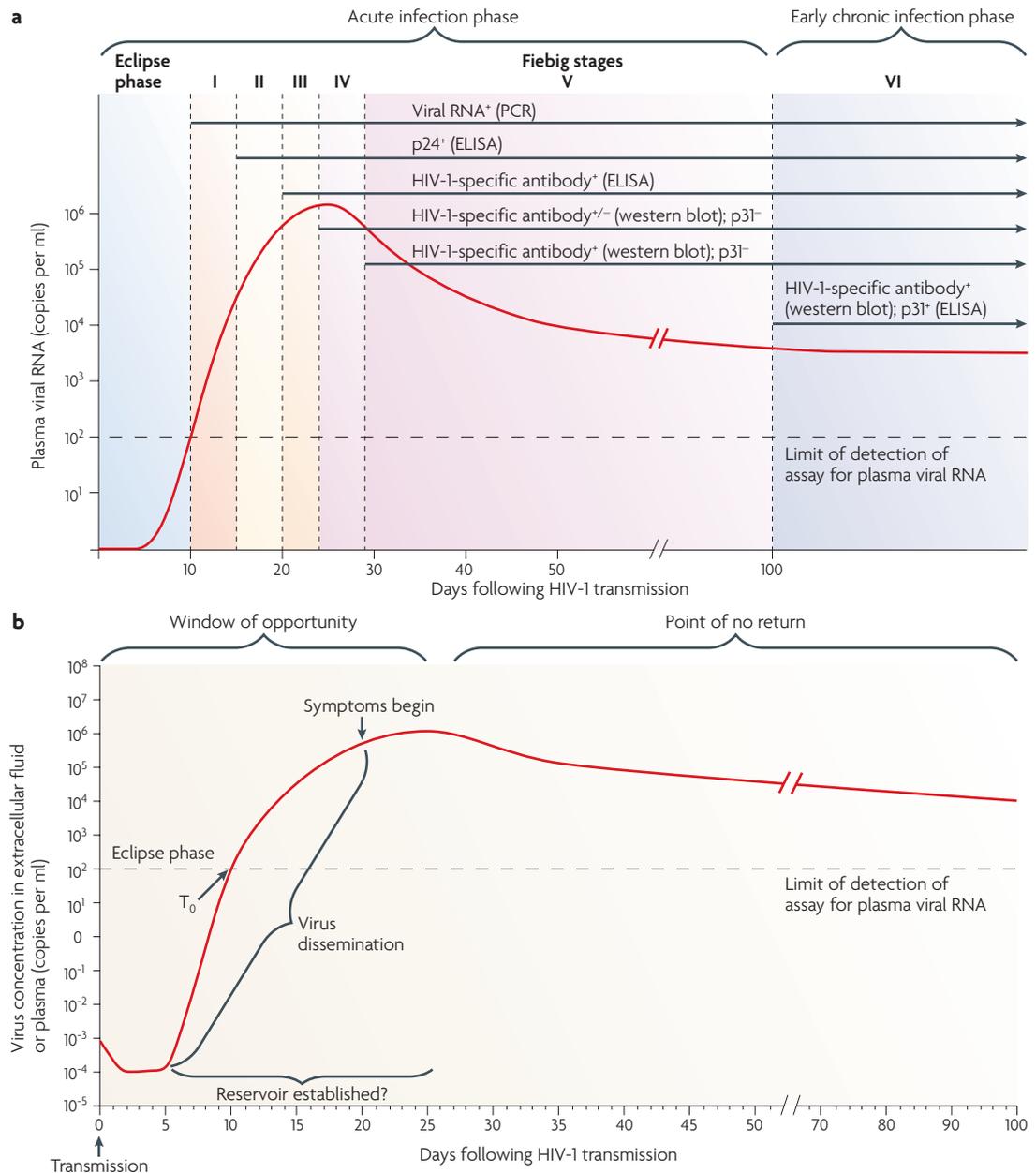
Figure 1 | **Definition of acute HIV-1 infection. a** | Recent analysis of samples from individuals early after infection with HIV-1 has revealed that the first weeks following infection can be divided into clinical stages that are defined by a stepwise gain in positivity for the detection of HIV-1 antigens and HIV-1-specific antibodies in diagnostic assays (in brackets)[82]. The time between infection and the first detection of viral RNA in the plasma is referred to as the eclipse phase. Plasma virus levels then increase exponentially, peaking at 21–28 days after infection, and this is followed by a slower decrease in plasma viral RNA levels. Patients can be categorized into Fiebig stages I–VI, which are based on a sequential gain in positive HIV-1 clinical diagnostic assays (viral RNA measured by PCR, p24 and p31 viral antigens measured by enzyme-linked immunosorbent assay (ELISA), HIV-1-specific antibody detected by ELISA and HIV-1-specific antibodies detected by western blot). Patients progress from acute infection through to the early chronic stage of infection at the end of Fiebig stage V, approximately 100 days following infection, as the plasma viral load begins to plateau. **b** | Fundamental events in acute HIV-1 infection. Following HIV-1 infection, the virus first replicates locally in the mucosa and is then transported to draining lymph nodes, where further amplification occurs. This initial phase of infection, until systemic viral dissemination begins, constitutes the eclipse phase. The time when virus is first detected in the blood is referred to as T_0; after this there is an exponential increase in plasma viraemia to a peak 21–28 days after infection. By this time, significant depletion of mucosal CD4^{+} T cells has already occurred. Around the time of peak viraemia, patients may become symptomatic and reservoirs of latent virus are established in cells that have a slower rate of decay than CD4^{+} T cells. The 'window of opportunity' between transmission and peak viraemia, prior to massive CD4^{+} T cell destruction and the establishment of viral reservoirs, is the narrow but crucial period in which an HIV-1 vaccine must control viral replication, prevent extensive CD4^{+} T cell depletion and curb generalized immune activation. Part **a** is modified, with permission, from REF. 12 © (2008) National Academy of Sciences, USA. Part **b** is modified from REF. 160.

Germinal centre
A highly specialized and dynamic microenvironment located in peripheral lymphoid tissues (for example, the spleen or lymph nodes). It is the main site of B cell maturation, leading to the generation of memory B cells and plasma cells that produce high-affinity antibody.
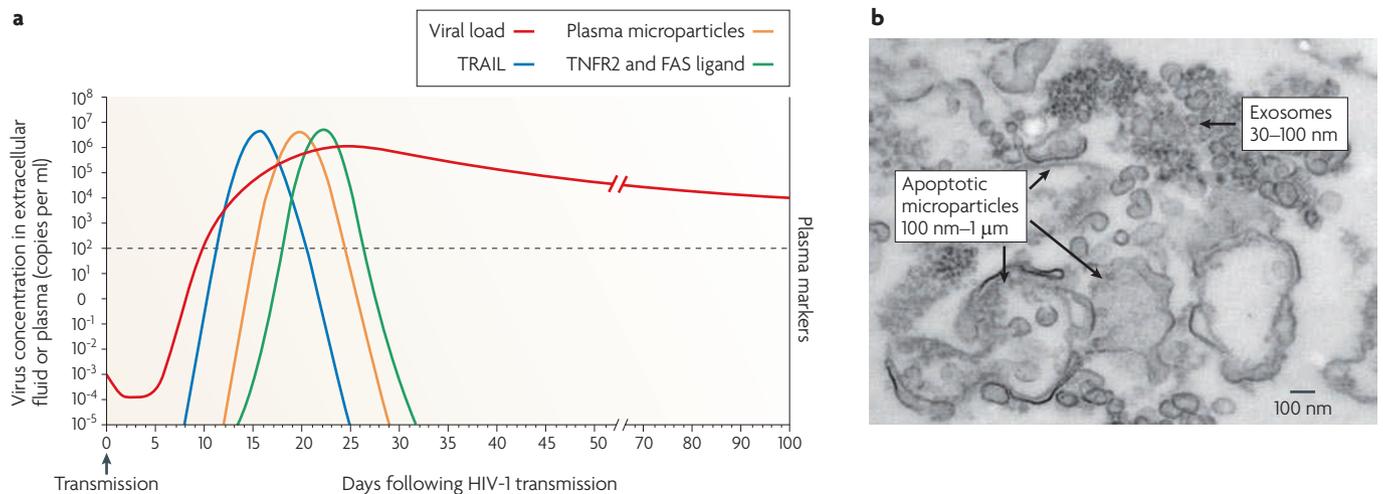
Figure 2 | **Early events in acute HIV-1 infection. a** | The timing of the appearance of soluble proteins and apoptotic microparticles in the blood during acute HIV-1 infection. An increase in the level of soluble tumour necrosis factor-related apoptosis-inducing ligand (TRAIL; also known as TNFSF10) in the plasma is the first evidence of infection-induced apoptosis and/or immune activation and it occurs before the peak in viraemia. This increase in TRAIL also coincides with the appearance of high levels of interferon-α (IFNα; see FIG. 3). Soon thereafter increases in the number of apoptotic microparticles are observed, followed by increased levels of soluble TNF receptor 2 (TNFR2; also known as TNFRSF1B) and soluble FAS ligand. A portion of the microparticles express CC-chemokine receptor 5, suggesting that they originate from cellular targets of HIV-1 infection. The appearance of these soluble components in the plasma early during HIV-1 infection probably represents a pathological rather than a protective cascade of events that is a cause of, or is associated with, virus-induced CD4+ T cell death. **b** | Electron micrograph of apoptotic microparticles and exosomes in the plasma from a patient with acute HIV-1 infection. There are ~700-fold more microparticles than virions present in acute HIV-1 infection. Part **a** is based on data from REF. 23. Part **b** is reproduced, with permission, from REF. 23 © (2008) American Society for Microbiology.

Establishing viral set point. At the point of peak viraemia the immune response has not affected the amino acid sequence of the virus, despite the extensive activation of innate immune cells (see below). Thereafter, the viral load decreases over 12–20 weeks to reach a more stable level, known as the viral set point[27–29] (FIG. 1). Virus diversification occurs during this decrease in viral load, and multiple escape mutants are selected under the pressure of adaptive immune responses that are first detectable just before peak viraemia[30–33]. In the absence of antiretroviral drug therapy (ART), the set point is maintained by a balance between virus turnover and the immune responses.

The death rate of infected cells has been calculated from decay curves of viraemia after ART initiation[34]. For most infected memory T cells, the half-life is less than a day[34]. However, other cell populations have slower rates of decay[35], and cell populations other than CD4+ T cells maintain latent pools of HIV-1 (REF. 36). Cells are probably latently infected within days of HIV-1 transmission and are unlikely to be removed by natural or vaccine-stimulated anti-HIV-1 immune responses, given that they cannot be eliminated by ART[37].

Immune activation. Activation of innate cells and B and T cells is a striking feature of acute HIV-1 infection of humans and SIV infection of rhesus macaques, and it persists to a varying degree into chronic infection. The dysregulation of immune cells is not limited to cells that are infected by, or are specific for, HIV-1 (REF. 38). Chronic immune activation is not observed in naturally SIV-infected sooty mangabeys, in which

the infections rarely progress to AIDS. This is despite high levels of virus replication and acute CD4+ T cell depletion[39], suggesting a role for immune activation in AIDS development. Indeed, there is a positive correlation between markers of CD8+ T cell activation and HIV disease progression[40–42].

Immune activation is associated with early and extensive apoptosis of B and T cells, leading to the release of apoptotic microparticles into the blood (FIG. 2), and increased expression of tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL; also known as TNFSF10) and FAS ligand (also known as CD95L), which kill bystander cells and are immunosuppressive[23]. The causes of HIV-associated immune activation established in early HIV-1 infection are not clearly defined[42,43]. Multiple related events (reviewed in REF. 44) probably contribute to such activation, including direct viral infection of immune cells, pro-inflammatory cytokine production by innate cells (which drives both direct and bystander activation of other immune cells), translocation of microbial products into the blood through damaged intestinal epithelium[21,22], loss of virally infected regulatory T (T_Reg) cells and chronic mycobacterial and viral co-infections.

Genetic control of HIV-1 set point
In contrast to other pathogens that have infected and selected humans for millennia, HIV-1 is a new pathogen to humans[45–47]. Therefore, the influence of the host's genetics on the immune response to HIV-1 infection may be more evident. The most dramatic finding in this

Regulatory T (T_Reg) cell
A type of CD4+ T cell that is characterized by its expression of forkhead box P3 (FOXP3) and high levels of CD25. T_Reg cells can suppress many types of immune responses.
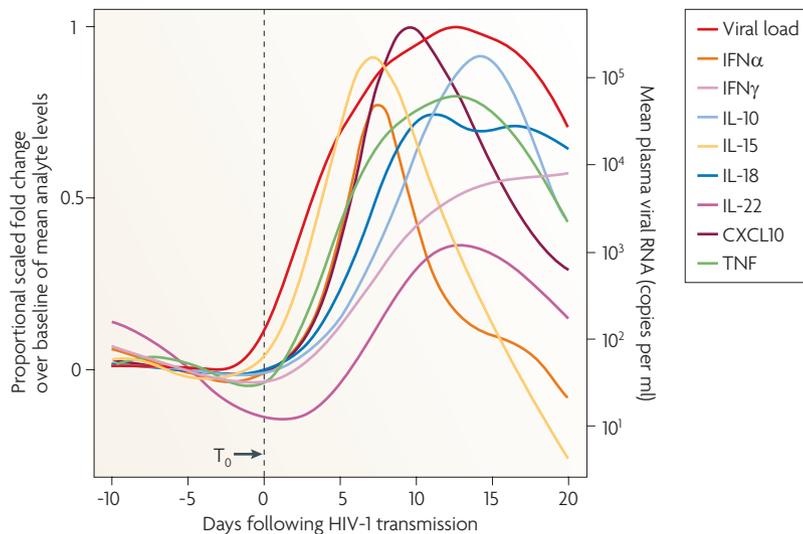
Figure 3 | **The cytokine storm in acute HIV-1 infection.** The relative kinetics of elevation of acute-phase proteins, cytokines and chemokines in the plasma during acute HIV-1 infection. There are two initial waves of cytokines: interleukin-15 (IL-15) and interferon-α (IFNα), followed by tumour necrosis factor (TNF), IL-18 and IL-10. CXCL10, CXC-chemokine ligand 10. Figure is reproduced, with permission, from REF. 58 © (2009) American Society for Microbiology.

Genome-wide association study
An approach that involves rapidly scanning single nucleotide polymorphism markers across the complete genomes of many individuals to find genetic variations associated with a particular disease.

Single nucleotide polymorphisms
(SNPs). Single nucleotide variations in genomic DNA sequences in which one of the four nucleotides is substituted for another. SNPs are the most frequent type of polymorphism in the genome and can be used to map genes connected by linkage disequilibrium.

Bw4 motif
The amino acid sequences at positions 77–83 in the α1 domain of HLA class I heavy chains. There are four Bw4 motif sequences, which differ only at positions 77 (Asn, Asp or Ser) and 80 (Ile or Thr). The Bw4 motif is involved in the recognition of certain HLA alleles by killer immunoglobulin-like receptors.

regard is that homozygosity for a 32 base pair deletion in *CCR5*, which abrogates its expression, protects almost completely from HIV-1 infection[48]. Furthermore, the HLA alleles HLA-B*5701, HLA-B*5703, HLA-B*5801, HLA-B27 and HLA-B51 are all associated with good control of the virus and a slower progression to AIDS[49], partly because the epitopes recognized by the T cells in these individuals are focused on conserved regions of the viral Gag protein (see below). A genome-wide association study[50] found a strong protective influence for a single nucleotide polymorphism (SNP) located 35 kilobases upstream of the HLA-C locus and confirmed the association of HLA-B57 with a low viral set point. This HLA-C-linked SNP may be associated with low-level expression of HLA-C[50], which might in turn affect T cell or natural killer (NK) cell function during HIV-1 infection. By contrast, some subtypes of HLA-B35 are associated with rapid disease progression, especially if homozygous[51], although the mechanism is not understood.

It has been shown that the expression of the killer immunoglobulin-like receptors KIR3DS1 and KIR3DL1 — which deliver activating and inhibitory signals to NK cells, respectively — delays progression to AIDS in individuals with HLA class I allotypes containing the 80Ile variant of the Bw4 motif[52], which are thought to be ligands for these receptors[53,54]. Expansion of NK cells that express KIR3DS1 and/or KIR3DL1 during acute HIV-1 infection has been observed but only if the HLA-B Bw4 80Ile motif is present[55], which is supported by *in vitro* data demonstrating that NK cells expressing KIR3DS1 control HIV-1 replication efficiently in HLA-B Bw4 80Ile-expressing target cells[56]. It is possible that KIR3DS1 mediates specific recognition of HIV-infected cells by NK cells, although the exact nature of the ligand is elusive. These observations probably reflect an influence

of interactions between KIR3DS1 and/or KIR3DL1 and HLA-B Bw4 80Ile on the development and/or functions of NK cells, and possibly CD8+ T cells, which may help to control viral set point.

Early innate immune responses to HIV-1
Acute-phase proteins and cytokines. Insight into the earliest systemic immune responses to HIV-1 infection has been gained by studying plasma donors who acquired HIV-1 infection. Frequent samples were taken before infection, through peak viraemia and sero-conversion[57,58]. Samples from different donors were aligned relative to the time that viral RNA was first detectable (100 copies per ml) (T_0). The first detectable innate immune response, occurring sometimes just before T_0, was an increase in the levels of some acute-phase proteins, such as serum amyloid A (H. Kramer and B. Kessler, personal communication). A further wave of acute-phase protein production coincided with a cytokine response (described below) and a rapid increase in plasma viraemia. The production of acute-phase proteins can be triggered by pro-inflammatory cytokines (such as interleukin-1 (IL-1)) and also by extrinsic factors such as lipopolysaccharide (LPS). LPS is detectable in the plasma during chronic infection with HIV-1 or SIV and may be derived from commensal bacteria that translocate from the gut lumen following depletion of HIV-1-infected intestinal CCR5+ T helper 17 cells[21,22,59]. Immunostaining of GALT biopsies collected from acutely infected patients showed higher levels of pro-inflammatory cytokines than healthy tissues[60].

As viraemia increases, so do the levels of cytokines and chemokines in the plasma (FIG. 3). Levels of IL-15, type I interferons (IFNs) and CXC-chemokine ligand 10 (CXCL10) increase rapidly but transiently. IL-18, TNF, IFNγ and IL-22 also increase rapidly but are sustained at high levels, whereas the increase in IL-10 is slightly delayed[58] (FIG. 3). Some of these cytokines have antiviral activity; for example, type I IFNs inhibit HIV replication in severe combined immunodeficient mice reconstituted with human lymphocytes[61]. Also, type I IFNs, IL-15 and IL-18 enhance innate and adaptive immune responses. However, the intense cytokine response during acute HIV infection may also promote viral replication and mediate immunopathology (discussed below).

The cellular sources of the acute-phase cytokines and chemokines during early HIV-1 infection have not been definitively identified, but probably include infected CD4+CCR5+ T cells, activated DCs[16], monocytes, macrophages[21], NK cells, NKT cells and, subsequently, HIV-specific T cells. The cytokine storm observed during early HIV-1 infection is much greater than that observed in acute hepatitis B and hepatitis C virus infections[58], indicating that a systemic cytokine response of this magnitude is not a pre-requisite for viral clearance. The intense cytokine response in acute HIV infection may instead fuel viral replication and mediate immuno-pathology (discussed below). High-level systemic cytokine responses during acute infections with avian influenza virus and severe acute respiratory syndrome-associated coronavirus are likewise associated with immunopathological consequences[62,63].

DCs. DCs are markedly reduced in number during acute HIV-1 infection[64] (N. Bhardwaj and P.B., unpublished observations). This rapid decline in circulating DCs, particularly plasmacytoid DCs (pDCs), may be due to activation-induced cell death or to the migration of activated DCs into lymph nodes, where an increase in DC numbers is observed[65,66]. *In vitro,* pDCs become activated by the binding of viral envelope proteins to CD4 expressed by the pDCs followed by virion endocytosis and by the triggering of Toll-like receptor 7 by viral RNA[67]. However, HIV-exposed conventional DCs do not become fully activated and show defective IL-12 production[68], which is consistent with the low levels of IL-12 observed during acute HIV infection[58]. In addition, HIV-exposed pDCs produce IFNα, which enhances adaptive immune responses. However, HIV-exposed pDCs also produce indoleamine 2,3-dioxygenase (IDO), which induces the differentiation of CD4+ T cells into T_{Reg} cells that might suppress HIV-specific immune responses[65,69,70]. Conventional DCs can prime virus-specific CD4+ and CD8+ T cell responses following *in vitro* exposure to HIV[71].

NK and NKT cells. As with most viral infections, NK cells and NKT cells become activated during acute HIV infection[56,72,73]. Prior to the peak in viraemia, blood NK cells proliferate and show enhanced activity when tested *ex vivo*[56]. The NK cell population expressing KIR3DS1 and/or KIR3DL1 expands during acute infection in individuals that also express HLA-B Bw4 80Ile[55]. NK and NKT cells can control HIV replication through cytolysis of virally infected cells and the production of antiviral cytokines and chemokines. In addition, they can interact with DCs and thereby influence T cell responses. HIV-1 has evolved a strategy to reduce the expression of ligands for NK cell receptors by infected cells[74]. This finding, and the clear role of KIR3D molecules in determining the viral set point[52,54], supports the involvement of NK cells in the control of HIV-1. However, the timing of NK cell antiviral effects remains uncertain. NK cells do not contribute to the selection of virus escape mutants before peak viraemia, although it is possible, but not proved, that they account for some of the unexplained mutations that appear together with those that are selected by early T cell responses as viraemia decreases to reach the set point[75]. Alternatively, the antiviral effects of NK (and/or NKT) cells might have a greater influence at later time points.

Implications for vaccine design. Can the protective potential of innate immune responses be harnessed by vaccination? Because NK cells share some characteristics with memory cells after their initial activation[76,77], it may be possible to prime their antiviral activity through vaccination. However, the activation of innate immunity should be attempted with caution, as innate immune responses can also be harmful. For example, induction of mucosal inflammatory responses by some microbicides has led to increased acquisition of HIV-1 infection (reviewed in REF. 78). Furthermore, as discussed earlier, activated DCs can transmit virus to CD4+ T cells

and, during the eclipse phase of infection, chemokines produced by pDCs can recruit susceptible CD4+ T cells to the foci of infection[16,79]. Immune activation induced by innate immune cells and the resulting production of pro-inflammatory cytokines and chemokines can promote HIV-1 replication. Type I IFNs and TNF also have pro-apoptotic effects and can thereby contribute to a loss of activated DCs and the bystander destruction of CD4+ T cells and B cells. The opposing effects of innate immune activation were highlighted in a study in which IL-15 was administered to treat acute SIV infection in rhesus macaques: NK cell and SIV-specific CD8+ T cell numbers were increased, resulting in fewer SIV-infected cells in lymph nodes, but the activation and proliferation of CD4+ T cells was enhanced and a higher viral load was established[80]. Therefore, vaccine-induced activation of innate immune responses will have to be thoroughly tested in the macaque SIV model and used with caution in humans.

Early T cell responses in HIV-1 infection

CD8+ T cell responses. A few studies have measured HIV-1-specific CD8+ T cell responses during early HIV-1 infection, before the first antibodies are detectable[30,31,33,81] (Fiebig stages I or II[82]). Similar to SIV infection in macaques, the first T cell responses to HIV-1 infection arise as viraemia approaches its peak, and the T cell response peaks 1–2 weeks later, as viraemia declines. The homogeneity of the founder virus at the time of the peak of viraemia[12–14] indicates that there is no immune-driven selection of escape mutants as viraemia increases.

Following the peak in the CD8+ T cell response, the virus sequence starts to change dramatically. Rapid selection of mutations occurs at discrete sites in the virus genome as viraemia declines to the viral set point[14,83] (FIG. 4). Detailed analysis of four patients during the very early stages of infection[75] indicated that most of the amino acid changes in the virus were selected by CD8+ T cells that recognize epitopes expressed by the founder virus but not by the escape mutant virus. Mutations in the viral envelope protein that were selected by neutralizing antibodies appeared later, at ~12 weeks. A minority of virus escape mutants were not associated with demonstrable T cell responses: a few mutations were probably reversions from the sequence of the transmitted virus that was selected by T cells in the patient's sexual partner; others may have been selected by antibody-dependent cell-mediated virus inhibition or by NK cells. Notably, T cell- and antibody-mediated selection of viral escape mutants rarely involved a single amino acid change in the epitope; most mutants involved multiple changes such that various mutants were 'tested' until the fittest were selected[75]. The first T cell-selected mutations could replace the original sequence of the founder virus within 10 days, and were then followed by sequential selection of escape mutations at different epitopes. This pattern continues throughout the course of HIV infection[75]. Changes in sequence could involve amino acids that are upstream of the T cell epitope and are probably important for antigen processing[84–86].

Figure 4 | Early T cell selection of virus escape mutations in acute HIV-1 infection. The virus escape mutations occurring in a single representative patient during acute HIV-1 infection. The changes in plasma virus load (**a**) and the emergence of amino acid changes (**b**) are shown. At the first time point, when the patient was virus positive but seronegative (Fiebig stage II), the founder virus (which was clade B) showed no evidence of immune selection. Thereafter there is an increasing number of selected sites at which the founder virus sequence is completely altered by, usually a cluster of, amino acid changes. Those marked in red were selected early (within 50 days from peak viraemia) by demonstrable CD8+ T cell responses. Those in purple were selected later by CD8+ T cells. Those in light green are single amino acid reversions to the clade B virus consensus sequence. Those in blue were mutations in V1 and V3 of the *env* gene selected by neutralizing antibodies. Those in grey were selected through undefined means, possibly by T cells, natural killer cells or antibodies. Yellow represents changes that co-varied with another mutation. LTR, long terminal repeat. Figure is based on data from REF. 75.

The earliest T cell responses are often specific for Env and Nef[75,87]. Responses to other viral proteins, including the conserved Gag p24 and Pol proteins, tended to arise during later waves of T cell responses and may be more important for maintaining the viral load at the set point than for controlling early viraemia[75,87,88]. Often, the first T cell responses decline rapidly when the escape mutations are selected, or they may decline through exhaustion[75,87]. The loss of T cells after virus mutation implies complete loss of the epitope and no tendency for the virus to revert to the original sequence because of loss of fitness.

The finding that escape mutants appeared so rapidly raises questions regarding the effectiveness of the early T cell response. A mathematical model has provided some answers[75]. The rapid loss of the founder virus sequence and its replacement by escape mutant viruses implies complete CD8+ T cell-mediated inhibition of virus production by infected cells. From the rate of loss of founder virus sequence, the fraction of cells killed per day was calculated to be 0.15–0.35 for the earliest T cell responses[75]. As a virus-infected cell has a lifespan of 1 day *in vivo*, this means that 15–35% of infected cells must be killed prematurely by a single T cell response, which must reduce virus production. Therefore, CD8+ T cells curb viraemia in acute HIV-1 infection. However, selection of escape mutants would

minimize this beneficial effect if the mutants were as fit as the founder virus and if the earliest responses were not immediately succeeded by new T cell responses to new (mutated) epitopes, which in turn may select further escape mutants[75] (FIG. 4). Ultimately, responding T cells target epitopes that are more highly conserved and in which escape occurs at a cost to the fitness of the virus. Such immunodominant responses to more highly conserved epitopes are more likely to result in a lower level of viraemia at the set point[89]. When a virus that has undergone such escape mutations is transmitted, its set point is also lower in the new host[90]. The level of set point viraemia is therefore influenced by the nature of the transmitted virus and the specificity of early CD8+ T cell responses. Immunodominant T cell responses to the more conserved immunodominant virus epitopes are likely to result in a lower viral set point[89].

CD8+ T cells are also important for the maintenance of viral set point. There have been many reports of virus escape mutations from around the time the set point is reached[30,32,84,85,91–99]. Using the same mathematical models as described earlier, CD8+ T cells are thought to make only a small contribution (killing 4–6% of virus-infected cells per day) to infected-cell death during chronic infection[100], the rest being due to virus cytopathicity or infected-cell activation. However, this may be an underestimate of the T cell contribution because of the fitness

costs of the escape mutations on the virus, such that mutant viruses grow more slowly than the founder virus. Some of the epitopes that are recognized by the T cells during later stages of infection are so highly conserved that the virus must undergo compensating mutations at other sites for escape to occur[75,94,97,98,101], which slows the outgrowth of the mutant viruses. The calculation is further confounded by the difficulty of simultaneous virus escape from more than one T cell response[13,85,91]. In contrast to the earliest stages of HIV-1 infection when the range of epitopes recognized by the T cell response is narrow, the later response is broad, often directed against more than 10 epitopes[102]. Responses to conserved epitopes are probably important in the long-term control of viral load, because patients with HLA-B27, HLA-B*5701, HLA-B*5703 or HLA-B*5801 that do well clinically have CD8+ T cells that recognize less-variable regions of the virus, particularly in Gag. The HIV-1 quasispecies in these patients do escape slowly during long-term infection, but each escape mutant incurs a proved fitness cost to the virus[101]. The time it takes for the first T cell responses to become targeted to conserved epitopes might be important in determining long-term control of viral infection[75,88,99]. It is not clear what features determine which CD8+ T cell epitopes will become immunodominant; it is clear that HLA type is important, but the precursor frequency of naive T cells that are specific for HIV proteins is also likely to be a factor that is probably influenced both by genetics and a history of previous (cross-reactive) antigen exposure. Vaccines could influence this.

The CD4+ T cell response. HIV-1 infects and significantly depletes memory CD4+ T cells[25,79], and HIV-1-specific CD4+ T cells are particularly susceptible to HIV-1 infection[103]. CD4+ T cell responses to HIV proteins have always been difficult to show, and there is a disparity between the measurements of CD4+ T cell responses to antigen when observing cytokine production versus proliferation[104]. Nevertheless, several epitopes for CD4+ T cells have been identified, particularly in Gag[105]. Expansion of HIV-specific CD4+ T cell responses occurs in acute HIV-1 infection, but such responses decline rapidly[106,107]; although, very early administration of ART, to control viraemia and prevent the killing of CD4+ T cells, can rescue strong HIV-1 CD4+ T cell responses[108,109]. However, even with the probably suboptimal help from the weakened CD4+ T cell repertoire, the first CD8+ T cell responses are strong, although their progression into long-term memory cells could be impaired. The rapid decline of CD8+ T cell responses observed after the founder epitope is eliminated from the virus in the plasma, owing to escape mutations[75], is consistent with the impaired long-term CD8+ T cell memory that has been observed in a model in which mice were depleted of CD4+ T cells[110].

Implications for vaccine development. The findings described above suggest a role for CD8+ T cells in the earliest immune control of acute HIV-1 infection. CD8+ T cells develop abnormally[111] and become dysfunctional as HIV-1 infection progresses (reviewed in REF. 112), but the early HIV-1-specific CD8+ T cell response seems to be functionally normal[109] (G. Ferrari, personal communication). Although not all the factors that contribute to a low virus set point and good long-term prognosis (without ART) are known, it is clear that CD8+ T cells are important components. If a vaccine cannot completely prevent infection, there should be a benefit from stimulating appropriate CD8+ T cell responses, as shown recently in the macaque SIV model[113]. An effective vaccine would need to stimulate CD8+ T cell responses to multiple epitopes, especially to those that are highly conserved. It would also be favourable to stimulate a broad T cell response that recognizes common variants of the founder virus epitope sequence, which would limit escape options[114,115].

Antibody responses during acute HIV-1 infection

Early neutralizing and non-neutralizing antibody responses. Antibodies that neutralize autologous virus develop slowly, arising ~12 weeks or longer after HIV-1 transmission[116–118]. Antibodies that show some degree of neutralization of heterologous virus eventually arise in ~20% of patients years after infection[117,119,120]. To determine the specificity and kinetics of antibody production after HIV-1 transmission and to understand why broadly reactive neutralizing antibodies are not made during acute HIV infection, it is important to study the earliest B cell responses to the transmitted virus[117,118].

Env-specific antibody responses to autologous, consensus Env epitopes were determined in the same plasma donor cohort as described earlier for innate immunity[57]. The first detectable B cell response was found to occur 8 days after T_0 in the form of immune complexes, whereas the first free antibody in the plasma was specific for Env glycoprotein (gp)41 and appeared 13 days after T_0. By contrast, the appearance of Env gp120-specific antibodies was delayed an additional 14 days, as was the production of other non-neutralizing Env-specific antibodies[57,118,121–123] (FIG. 5; TABLE 1). The first HIV-1-specific IgA responses in mucosal secretions, which were detected within the first 3 weeks after T_0, also recognized gp41 during acute HIV infection (N. L. Yates and G.D.T., unpublished observations). A study that applied mathematical modelling to early viral dynamics indicated that the initial gp41-specific IgG and IgM responses did not significantly affect the early dynamics of plasma viral load[57]. These acute gp41- and gp120-specific antibodies did not select escape mutations, indicating that these early arising antibodies are ineffective against HIV-1. Similar analyses of the effect of the initial immune complexes and gp41-specific IgA responses on viral dynamics are needed to understand the interplay between the initial host antibody responses and virus replication. It is not known why the initial antibody response to Env is non-neutralizing; it may relate to the immunodominance of denatured or non-functional Env forms[124,125]. The first antibodies to induce escape mutants are autologous-virus-neutralizing antibodies that develop ~12 or more weeks after transmission (TABLE 1). Fc receptor
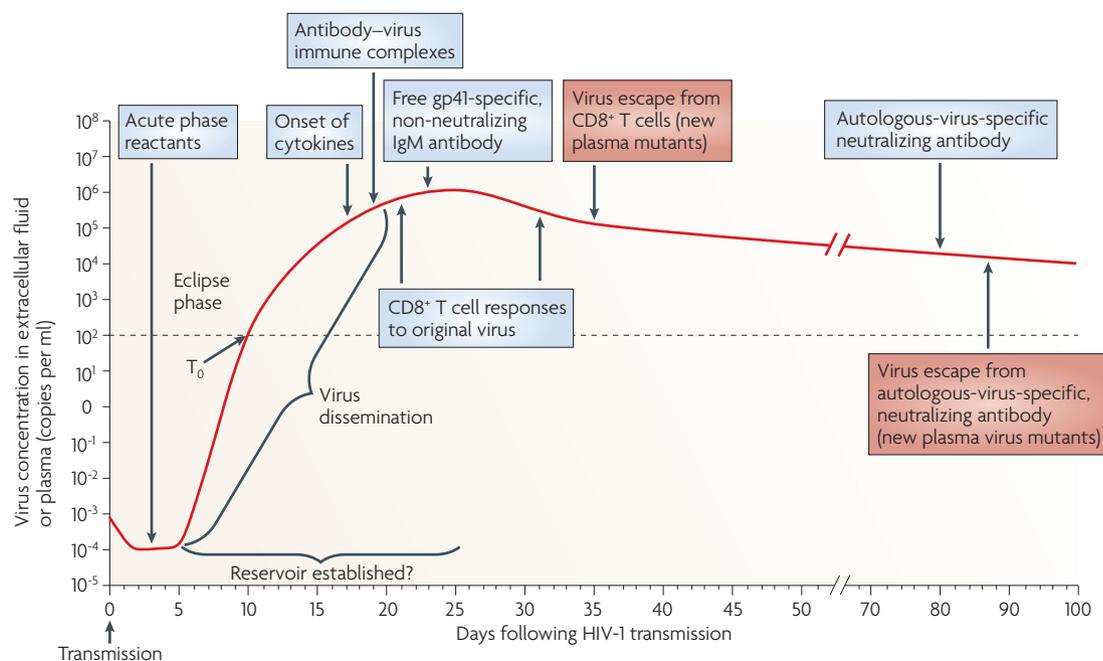
Figure 5 | **Composite alignment of the earliest innate and adaptive immune responses detected after HIV-1 transmission.** The first systemically detectable immune responses to HIV-1 infection are the increases in levels of acute-phase proteins in the plasma, which are observed when virus replication is still largely restricted to the mucosal tissues and draining lymph nodes (eclipse phase). When virus is first detected in the plasma (T_0), broad and dynamic increases in plasma cytokine levels are also observed. Within days, as plasma viraemia is still increasing exponentially, the first antibody–virus immune complexes are detected. Expansion of the earliest HIV-1-specific CD8^{+} T cell responses also commences prior to peak viraemia, followed by detection of the first free glycoprotein (gp)41-specific but non-neutralizing IgM antibodies. Complete virus escape from the first CD8^{+} T cell responses can occur rapidly, within 10 days of T cell expansion. By this time, viral reservoirs exist, possibly becoming established within days of infection. The earliest autologous-virus-neutralizing antibodies are detected around day 80 following infection, as viral loads are still declining prior to the onset of the viral set point. Antibody escape virus mutants emerge in the plasma within the following week.

(FcR)-mediated and complement-associated anti-HIV effector functions have also been reported during primary infection[126–128]; however, further studies are required to define their role and capacity to select escape mutations.

The range of epitopes bound by the first (specific to autologous virus) neutralizing antibodies in HIV-1 clade C infection is narrow and epitopes are often restricted to certain virus isolates[129]. Similar to infections with clade B HIV-1, the initial autologous-virus-neutralizing antibodies induced in clade C infections are induced with similar kinetics and are usually specific only for the initially transmitted Env variant[116,117]. Although the autologous-virus-neutralizing antibody response can control the virus quasispecies present when these antibodies appear in infections with HIV-1 clade B or C viruses, the narrowness of the response allows rapid viral escape[116,117] (K. Bar and G. Shaw, personal communication).

Broad-specificity, neutralizing antibodies to conserved Env regions are rare. Interestingly, antibodies specific for the conserved regions of HIV-1 Env — such as the carbohydrate epitope recognized by the unique broad-specificity neutralizing monoclonal antibody 2G12 (REF. 130), the CD4-binding site recognized by the

monoclonal antibody 1b12 (REF. 131) and the membrane-proximal region recognized by the monoclonal antibodies 2F5, Z13 and 4E10 (REFS 132–134) — are rarely generated during HIV-1 infection; when they do occur, they develop only after ~20–30 months of infection[57,119,120,135]. These observations indicate that both genetic factors and maturation of the antibody response to HIV-1 are necessary for the generation of this rare, late, broad-specificity, neutralizing antibody response. Affinity maturation through somatic hypermutation may be crucial for the generation of these neutralizing antibodies and may be delayed because of impaired CD4^{+} T cell help. Because the 2F5, 4E10 and 1b12 monoclonal antibodies have long hydrophobic complementarity-determining region 3 (CDR3) sequences and show polyreactivity for autologous molecules, it has been suggested that B cell regulatory mechanisms such as self tolerance may control their production[136].

Early damage to mucosal B cell generative microenvironments. Acute HIV-1 infection profoundly affects blood and tissue B cells[123]. HIV-1 induces early class switching in polyclonal B cells and is associated with marked increases in the number of blood and tissue memory B cells and plasma cells, as well as a decrease in the number of naive B cells[123]. In the mucosal B cell

Class switching
The somatic-recombination process by which the class of immunoglobulin is switched from IgM to IgG, IgA or IgE.

Table 1 | **Env-specific antibody responses in acute HIV-1 infection**

Antibody specificity	Time of onset after transmission (days)
gp41	23
gp120	38
Non-neutralizing to CD4-binding site, MPER and CD4-inducible epitopes	40–70
Autologous-virus neutralizing antibodies	Earliest ~84
Broad-specificity and neutralizing to CD4-binding site, carbohydrate and MPER	Not usually made, but when they are they arise ~30 months after transmission in chronic infection

gp, glycoprotein; MPER, membrane-proximal external region.

inductive microenvironments, such as Peyer's patches, where HIV and SIV replicate at high levels during acute infection[137–139], both HIV-1 (REFS 123,139) and SIV[140] can induce the lysis of follicular B cells, massive B cell apoptosis and loss of ~50% of germinal centres within the first 80 days of infection. Early loss of germinal centres may result in defects in the ability to rapidly generate high-affinity HIV-1 antibodies and lead to a delay in the induction of autologous-virus-neutralizing antibodies.

Implications for vaccine design. The finding that the generation of potentially protective antibodies is delayed until after initial control of viraemia ~12 weeks after transmission and then focused on only a few epitopes implies that it will be important to develop a vaccine that primes a very early and broad antibody response that targets multiple neutralizing epitopes for effective control of early viral expansion; the natural process is too little, too late. The early perturbations to B cells by the virus similarly indicate the need for a vaccine that either has high levels of durable protective antibody responses or primes in order to induce a rapid secondary response. The rarity of broad-specificity, neutralizing antibody responses to conserved epitopes in Env emphasizes the need to search for and find those small B cell subsets that can make broad-specificity, neutralizing antibodies: immunogens and adjuvants are needed that target those specific B cells.

Outlook

A clear picture of the earliest immune responses to HIV-1 (FIG. 5) has major implications for HIV-1 prevention in general and for vaccine design. After transmission, there is probably only a 5–10 day window during the eclipse phase in which the virus-infected cells could be eradicated, before the virus spreads widely and integrates to generate long-lasting and non-eradicable reservoirs of latent virus. True sterilizing immunity can be attained only if the virus is prevented from infecting any host cells. This could be achieved only through broad-specificity neutralizing antibodies that are already present in the plasma and at mucosal sites before virus transmission. In support of this hypothesis, it has been shown that local application, or intravenous injection, of neutralizing monoclonal antibodies against SIV in macaques is protective against subsequent challenge with the virus[141–144].

Peyer's patches
Specialized lymphoid follicles localized in the submucosa of the small intestine and appendix.

Antibody-dependent cell-mediated cytotoxicity (ADCC). A cytotoxic mechanism by which an antibody-coated target cell is directly killed by a leukocyte that expresses FcRs, such as an NK cell, macrophage or neutrophil.

If neutralizing antibodies cannot be generated in sufficient quantity, affinity and breadth, other immune mechanisms could abort the infection by attacking the founder virus and/or the first infected cells. CD8+ T cell-mediated killing, antibody-mediated mechanisms dependent on FcRs (including antibody-dependent cell-mediated cytotoxicity (ADCC)), NK cell-mediated lysis and β-chemokine release all have the potential to prevent early infection. However, to prevent infection these effector mechanisms would have to be ready primed, as there is not time to activate and expand central memory CD8+ T cells[145], for example, before chronic infection is established.

Harnessing NK cells and NKT cells might be an effective strategy to control the increase of virally infected cells during the eclipse phase or during the increase in viraemia of early HIV-1 infection. Although it may be hazardous to induce chronic hyperactivation of these cells as a means to inhibit virus infection, it may be possible to immunize subjects with HIV-1 antigens such as peptides that specifically expand potentially protective NK and NKT cell subpopulations, thereby altering the cell repertoire to contain a higher proportion of protective NK cells[76,77].

The modest protection offered by the vaccines used in the recent RV144 clinical trial carried out in Thailand with volunteers at low risk of HIV-1 infection[146] (BOX 1) may be an example of weak immune responses combining to raise the threshold for infection — a rare event.

Once infection starts to spread, enhancing the natural containment processes might be the only immunological option to benefit infected individuals. CD8+ T cell responses, which are clearly effective in reducing the peak viraemia during acute infection, could be enhanced through vaccination by increasing their breadth of epitope recognition so that, rather than mediating sequential responses to single epitopes, there would be a simultaneous multi-epitope-specific CD8+ T cell response to the virus[113,147,148]. Focusing this response on conserved epitopes, for which escape incurs a fitness cost to the virus, would be desirable. Strategies for enhancing or preserving CD4+ T cell help would also be of benefit for supporting the CD8+ T cells. However, it is important to recognize that CD8+ T cells are highly sensitive to single amino acid variation in epitope peptides[75,149], so even minor mismatches between vaccine-encoded epitopes and viral epitopes could be a serious problem and could diminish the effectiveness of any vaccine-stimulated T cell response.

If a vaccine can induce greater breadth in early T and B cell responses to HIV-1 than occurs naturally during acute infection, then the use of a combination of protective epitopes in a preventative vaccine may control the early dissemination of HIV-1, resulting in a lower viral set point and better long-term immune control. Preliminary unpublished results with experimental vaccines that include multiple common variants of HIV-1 proteins such as Gag (mosaic vaccines) have been shown to enhance the breadth and magnitude of T cell responses in animal studies[150–152]. This approach and other novel strategies for expanding the breadth of induced Env-specific B cell responses are also central to improving the prospects of vaccine success.

It can be thought to be good news that most HIV-1 transmissions that result in productive infection are mediated by only one virion, indicating a vulnerability of the virus to immune attack during the eclipse phase. This suggests that a well-designed vaccine strategy might have a chance of achieving good (if not perfect) control around the time of acute peak viraemia, preventing the onset of damaging chronic immune activation and damage to generative immune cell environments. However, vaccine strategies must be developed that potentiate what is clearly a qualitatively and quantitatively insufficient immune response in the first few weeks of HIV-1 infection. It is hoped that both the innate and adaptive arms of the immune system can be harnessed to develop an HIV-1 vaccine that ensures that adequate immune protection is in place before transmission, enabling earlier, broader and more effective secondary responses for preventing or controlling acute HIV-1 infection.

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DATABASES
UniProtKB: http://www.uniprot.org
APOBEC3F | APOBEC3G | CCR5 | CD4 | CD21 | CXCL10 | DC-SIGN | IDO | IL-10 | IL-15 | IL-18 | IL-22 | KIR3DL1 | KIR3DS1 | serum amyloid A | TNF

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