

# Prospects for an Effective T Cell-Based Immunoprophylaxis against Mother-to-Child Transmission of HIV-1

( HIV-1 / paediatric vaccine / cytotoxic T cells )

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**Abstract.** Globally, more than 2000 children under 15 years of age are infected with HIV-1 every day. Some of these infections occur *in utero*, but the majority of children become infected at delivery and after birth through breast-feeding. While success of antiretroviral therapy dramatically decreased mother-to-child transmission in developed countries, antiretroviral drugs are not yet widely available and bottle-feeding is not an option in economically impoverished countries, where burden of HIV-1 infections is the highest. There, effective accessible HIV-1 vaccines limiting spread of HIV-1 in adults and preventing infection of neonates through breast-feeding are urgently needed. For infant vaccines, given the difficulties in inducing widely cross-reactive HIV-1-neutralizing antibodies, effort has now shifted towards elicitation of cell-mediated immunity, likely in a combination with passively infused neutralizing antibodies and/or chemoprophylaxis. This review discusses prospects of the T-cell approach for development of a paediatric HIV-1 vaccine.

An estimated total of 60 million people have become infected with human immunodeficiency virus type 1 (HIV-1), of whom some 22 million have died since the first report of acquired immunodeficiency syndrome (AIDS) in 1981 and its subsequent recognition as a novel disease (UNAIDS, 2001). Twenty years later, HIV-1 continues to spread particularly in developing countries in a largely uncontrolled manner. More than 60% of the world's HIV-1-infected people live in Africa and about a half of the infected adults are women of child-bearing age among whom AIDS is now the leading cause of death. In larger urban centres, up to 40% of pregnant women attending city clinics are seropositive and, without any intervention, about one third of the children born to these mothers become infected (Datta et al., 1994). Although approximately half of mother-to-

child transmissions (MTCT) are due to a prolonged breast-feeding (Van de Perre et al., 1991; Ekpini et al., 1997; Richardson et al., 2003a; Rousseau et al., 2003), for social, practical and health reasons, bottle-feeding is frequently not an option. Bottle-fed babies of infected mothers have a higher morbidity and mortality due to increased exposure and susceptibility to other infections (Mbori-Ngacha et al., 2001). Although antiretroviral therapy can significantly reduce risk of MTCT, the drugs have to be administered at birth and maintained throughout the whole period of breast-feeding, and their effectiveness might be compromised by resistant mutants. Furthermore, long-term effects of antiretroviral drugs on child's normal development are unknown. Thus, the best hope for protecting newborns in developing countries against HIV-1 is development of safe, effective, accessible prophylactic vaccines which would both reduce adult burden of infection and protect neonates against vertical HIV-1 transmission. While 60 different vaccine candidates have been or are being tested in about 150 individual human trials to date, very few of them have included children in their clinical trials (Safrit, 2004). Therefore, there is a need for safe, but accelerated testing of new vaccine approaches in children.

## Vaccine protection against HIV-1

A successful prophylactic vaccine should train host's defences to recognize HIV-1 antigens and induce a sustained level of immunological memory, which would give the host a sufficient head-start in the race against the incoming virus. Protection would be provided in one of three possible scenarios. Ideally, a vaccine should induce (i) a sterilizing immunity, thus preventing HIV-1 from infecting host cells. Although sterilizing immunity remains the ultimate aim of AIDS vaccine research, it will be extremely difficult to achieve and maintain. In fact, sterilizing immunity may not exist, because a limited infection of host cells might be unavoidable. However, even non-sterilizing immunity would be beneficial if it either (ii) allowed a detectable, but only transient replication of HIV-1 after which the virus is cleared from the body, or (iii) kept levels of HIV-1 replication so low that there is no transmission to other people and no progression to AIDS. Though not

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Abbreviations: AIDS – acquired immunodeficiency syndrome, CTL – cytotoxic T lymphocytes, HIV-1 – human immunodeficiency virus type 1, MTCT – mother-to-child transmission(s).

ideal, the latter might be similar to HIV-2 infection, which does not progress to disease in the majority of infected people (Marlink et al., 1994). In countries where drug treatments are not readily or reliably available, vaccines that do not prevent infection but prolong its asymptomatic phase may still be of a great benefit. Such vaccines are also likely to lower the transmission rate through increasing the threshold of infection.

An effective HIV-1 vaccine may have to stimulate both neutralizing antibodies and cell-mediated immune responses and do so both systemically and at mucosal sites. Seven broadly cross-neutralizing human monoclonal antibodies have been isolated, which could provide sterilizing immunity in monkey challenge models when passively infused preferentially in a combination (Ferrantelli and Ruprecht, 2002). However, all efforts to induce these antibody specificities by active immunization have so far failed (Burton et al., 2004a,b). Nevertheless, induction of HIV-1-neutralizing antibodies is in principle possible and remains an important goal of the HIV-1 vaccine development. On the other hand, a few new technologies capable of inducing high levels of circulating virus-specific CD8<sup>+</sup> cytotoxic T lymphocytes (CTL) in animal models are emerging (Hanke, 2003) and an increasing number of these are entering phase I and II clinical trials in adults, the results of which will become available in the coming years. Whether antibodies and CTL can be induced efficiently by a 'single' vaccine, or a combined approach inducing antibodies and CTL separately will be required, remains to be seen.

### The role of CTL

CTL recognize short 8- to 10-amino acid-long HIV-1-derived peptides presented by autologous major histocompatibility complex (MHC, in humans called HLA) molecules through their T-cell receptors. Recognition of these MHC-peptide complexes in the context of the right accessory signals stimulates naive and precursor memory CTL to proliferate and express effector functions. These include induction of apoptosis in HIV-1-infected cells, which limits the production of new HIV-1 virions, and production of soluble factors that can suppress HIV-1 replication. The advantage of CTL over antibodies is that the recognized peptides can originate from both surface and inner structural and non-structural HIV-1 proteins. The disadvantage is that CTL cannot prevent the first wave of cell-free HIV-1 from infecting host cells. However, once CTL become stimulated by antigen-presenting cells, they produce soluble factors relatively quickly that can, at least *in vitro*, prevent HIV-1 from entering cells (Zhang et al., 1996; Price et al., 1998; Wagner et al., 1998; Stranford et al., 1999). So under favourable circumstances, CTL may prevent HIV-1 from spreading and establishing a gener-

alized infection by eliminating the initial small number of HIV-1-infected cells.

A central role for CTL in the control of HIV-1 infection is supported by a number of experimental and natural history data. CTL responses were detected in one third to one half of exposed, but seronegative subjects such as commercial sex workers whose cells were fully susceptible to infection with HIV-1 (Rowland-Jones et al., 1993; Pinto et al., 1995; Rowland-Jones et al., 1995; Rowland-Jones et al., 1998; Kaul et al., 2000), infants born to HIV-1-infected mothers (Cheynier et al., 1992; Rowland-Jones et al., 1993; Aldhous et al., 1994; De Maria et al., 1994; Wasik et al., 1997), health care workers occupationally exposed to HIV-1-contaminated body fluids (Pinto et al., 1995), haemophiliacs who received contaminated factor VIII preparations (Gibbons et al., 1990) and sexual partners of known HIV-1-infected subjects (Mazzoli et al., 1997; Goh et al., 1999). Although the precise mechanism of CTL induction is not well understood, one study found in two out of 10 studied subjects persisting HIV-1 DNA at levels at least 1000 times below the detection limit of conventional assays (Zhu et al., 2003). In infected individuals, CTL fail to clear HIV-1 from the body; however, their appearance and not that of neutralizing antibodies correlates with the control of the primary viraemia (Borrow et al., 1994; Koup et al., 1994; Kent et al., 1997). HIV-1-specific CTL are detected in all HIV-1-positive asymptomatic individuals (Walker et al., 1987; Harrer et al., 1996; Ogg et al., 1998) and their function decreases with the overall collapse of the immune system reflected by development of opportunistic infections and AIDS. Depletion of CD8<sup>+</sup> T cells from monkeys before or after a simian immunodeficiency virus (SIV) challenge increases the viral load, while their subsequent reappearance negates the effect and brings viraemia back to the pre-treatment level (Matano et al., 1998; Jin et al., 1999; Schmitz et al., 1999). Further support for the role of CTL comes from epidemiological studies implicating HLA in driving HIV-1 evolution (Moore et al., 2002; Yusim et al., 2002), as well as associations of rare HLA types with lower viraemia (Altfeld et al., 2003) and HLA heterozygosity with a better clinical outcome (Carrington et al., 1999).

To maximize any vaccine-induced CTL protection against HIV-1, virus replication must be controlled early after exposure. The more is HIV-1 allowed to replicate, the more variants it generates and the more it damages the immune system. Thus, to deflect the HIV-1 infection, sufficient numbers of resting, cycling and/or effector CTL need to be induced and maintained. This may or may not require persistent vaccine stimulation, regular re-vaccinations or repeated exposure to HIV-1 as suggested by initially protected, but after-break infected commercial sex workers (Kaul et al., 2001). The specificity of CTL may play a significant role, as recognizing early HIV-1

proteins of preferably transmitting virus/clade may give CTL just enough of a head-start to tip the fine balance between HIV-1 replication and suppression in their favour (Gallimore et al., 1995; Cafaro et al., 1999; Osterhaus et al., 1999; Allen et al., 2002; Baalen et al., 2002). Broad recognition of multiple CTL epitopes (Goulder et al., 1997; McMichael, 1998; Carrington et al., 1999; Kaslow et al., 2001) and functionally conserved protein regions (Kelleher et al., 2001) would make it harder for HIV-1 to escape. Furthermore, CTL have to kill HIV-1-infected target cells efficiently and this requires their 'full' functionality. HLA-peptide tetrameric complexes demonstrated that specific T cells could be present, but anergized (Zajac et al., 1998), deficient in perforin (Andersson et al., 2002) or not fully differentiated (Champagne et al., 2001; Appay et al., 2002). However, all the immune impairments induced by HIV-1 infection do not affect prophylactic vaccination, which stimulates immunity in healthy HIV-1-uninfected individuals with an intact immune system. Finally, CTL do not act alone. Their action is supported by CD4<sup>+</sup> T-helper, dendritic, B and other cells involved in the intricate network of interactions leading to an efficient CTL response.

### HIV-1 immune escape

HIV-1's high replication and mutation rates generate a population of highly diverse 'quasispecies'. CTL, just like antibodies, exert a strong selective pressure, which suppresses sensitive and allows perhaps less virulent, but also less targeted HIV-1 variants to overgrow. Escaping antibodies is easier than escaping CTL, because the surface envelope has a bigger freedom to mutate without losing its function (up to 35% of amino acids can differ among clades) compared to more constrained inner proteins. Nevertheless, CTL escape remains the key to HIV-1 survival and eventual victory (Klenerman et al., 2002; O'Connor et al., 2002). For a non-sterilizing vaccine protection, there may be an opportunity for HIV-1 to escape even after a prolonged period of control, especially if the immune responses are focused on a small number of CTL epitopes (Barouch et al., 2002). However, escape mutations do not become fixed, but can revert back following a transmission into a non-selective environment in an HLA-diverse human population. Thus, HIV-1 does not become invisible to CTL through accumulation of escape mutations (Friedrich et al., 2004; Leslie et al., 2004).

It is not known what level and breadth of memory T cells will an effective vaccine need to elicit to protect against or significantly reduce the chances of HIV-1 infection. This can be only determined by efficacy trials in humans involving in adult population thousands of high-risk volunteers and a considerable expense. For justification of the first efficacy trials, a minimal level of vaccine-induced HIV-1-specific memory T cells will be informed by macaque studies (Barouch et al., 2000),

CTL levels in seronegative HIV-1-exposed individuals (Rowland-Jones and McMichael, 1995) and vaccine ability to induce immune responses capable of imposing a selective pressure on break-through HIV-1 infections in phase IIB proof-of-principle clinical trials in high-risk volunteers.

### The paediatric angle

Compared to adults, infants in the first two years of life have higher HIV-1 load, higher CD4 cell counts in the blood, lower HIV-1-specific CTL responses and faster progression to AIDS (Mo et al., 1998; Luzuriaga and Sullivan, 2002). The critical issues for paediatric HIV-1 vaccines and vaccine preventing MTCT in particular are whether or not neonates have a mature enough immune system so that they are capable of mounting a T-cell response to vaccination soon after birth, and how long it takes for these protective responses to develop. Although there was a report of defective natural killer cell-mediated cytotoxicity in newborn babies (Ziegner et al., 1999), there is ample evidence for a mature CD8 T-cell responsiveness. In addition to exposed uninfected infants (Cheynier et al., 1992; Rowland-Jones et al., 1993; Aldhous et al., 1994; De Maria et al., 1994; Wasik et al., 1997), HIV-1-specific T-cell responses are considered to be an important marker of early HIV-1 infection (Luzuriaga et al., 1995; Pikora et al., 1997; Spiegel et al., 2000; Scott et al., 2001) and were shown to delay infant progression to disease (Buseyne et al., 1998; Wasik et al., 2000; Buseyne et al., 2002). Evidence for early protective T-cell responses also comes from the findings that HIV-1-exposed infants were more likely to become infected with an HIV-1 variant that had escaped mother's immune responses to shared HLA types (Wilson et al., 1999; Goulder et al., 2001). Furthermore, even the human foetus is capable of making mature responses to a specific pathogen (Marchant et al., 2003), although this needs to be confirmed for HIV-1. Studies in non-human primates demonstrated development of a complete repertoire of properly organized antigen-presenting cells, T cells, and B cells as early as the second trimester of foetal life (Otsyula et al., 1996; Makori et al., 2003; Van Rompay et al., 2003) and showed that vaccination *in utero* and at birth can affect the frequency of transmission (Makori et al., 2003; Van Rompay et al., 2003). In any case, newborn vaccinations may have to be combined with chemoprophylaxis or passive antibody immunoprophylaxis, which would prevent HIV-1 infection until active protective immunity is established.

The natural history of HIV-1 infection and responsiveness to vaccinations differ in adults and young children/neonates to such an extent that parallel adult and paediatric clinical trials are required (Richardson et al., 2003b). Consequently, some paediatricians feel that phase I safety in adults are sufficient preliminary data for vaccine trials in children and call for abandoning the

requirement to demonstrate prior efficacy in adults. This requirement not only causes delays in the children vaccine development, but may miss vaccines ineffective in adults, but effective in children. Moreover, because of the large number of infants born at risk of HIV-1 infection and high rate of transmission within the first 6 months of life due to breast-feeding, immunoprophylaxis studies in newborns might reach conclusive endpoints in 18 months and therefore accelerate vaccine development (Safrit et al., 2004). Needless to say that complex ethical issues have to be resolved.

## Conclusion

Focus on T-cell induction is a relatively new concept in vaccinology as the majority of today's vaccines work through induction of antibodies. Although there are encouraging data from animal models, most of the novel T-cell vaccine approaches are only now entering clinical trials in adult humans. These are combinations of subunit genetic vaccines vectored by DNA, recombinant viruses and bacteria, or formulated as adjuvanted proteins and peptides. Although translating these data into children may add some extra years, there is a cause for a cautious optimism. Let's not forget that most of the vaccine success stories to date come from children vaccinations.

## References

- Aldhous, M. C., Watret, K. C., Mok, J. Y., Bird, A. G., Froebel, K. S. (1994) Cytotoxic T lymphocyte activity and CD8 subpopulations in children at risk of HIV infection. *Clin. Exp. Immunol.* **97**, 61-67.
- Allen, T. M., Mortara, L., Mothe, B. R., Liebl, M., Jing, P., Calore, B., Piekarczyk, M., Ruddersdorf, R., O'Connor, D. H., Wang, X., Wang, C., Allison, D. B., Altman, J. D., Sette, A., Desrosiers, R. C., Sutter, G., Watkins, D. I. (2002) Tat-vaccinated macaques do not control simian immunodeficiency virus SIVmac239 replication. *J. Virol.* **76**, 4108-4112.
- Altfeld, M., Addo, M. M., Rosenberg, E. S., Hecht, F. M., Lee, P. K., Vogel, M., Yu, X. G., Draenert, R., Johnston, M. N., Strick, D., Allen, T. M., Feeney, M. E., Kahn, J. O., Sekaly, R. P., Levy, J. A., Rockstroh, J. K., Goulder, P. J., Walker, B. D. (2003) Influence of HLA-B57 on clinical presentation and viral control during acute HIV-1 infection. *Aids* **17**, 2581-2591.
- Andersson, J., Kinloch, S., Sonnerborg, A., Nilsson, J., Fehniger, T. E., Spetz, A. L., Behbahani, H., Goh, L. E., McDade, H., Gazzard, B., Stellbrink, H., Cooper, D., Perrin, L. (2002) Low levels of perforin expression in CD8+ T lymphocyte granules in lymphoid tissue during acute human immunodeficiency virus type 1 infection. *J. Infect. Dis.* **185**, 1355-1358.
- Appay, V., Dunbar, P. R., Callan, M., Klenerman, P., Gillespie, G. M., Papagno, L., Ogg, G. S., King, A., Lechner, F., Spina, C. A., Little, S., Havlir, D. V., Richman, D. D., Gruener, N., Pape, G., Waters, A., Easterbrook, P., Salio, M., Cerundolo, V., McMichael, A. J., Rowland-Jones, S. L. (2002) Memory CD8+ T cells vary in differentiation phenotype in different persistent virus infections. *Nat. Med.* **8**, 379-385.
- Baalen, C. A., Guillon, C., Baalen, M. v. M., Verschuren, E. J., Boers, P. H., Osterhaus, A. D., Gruters, R. A. (2002) Impact of antigen expression kinetics on the effectiveness of HIV-specific cytotoxic T lymphocytes. *Eur. J. Immunol.* **32**, 2644-2652.
- Barouch, D. H., Kunstman, J., Kuroda, M. J., Schmitz, J. E., Santra, S., Peyerl, F. W., Krivulka, G. R., Beaudry, K., Lifton, M. A., Gorgone, D. A., Montefiori, D. C., Lewis, M. G., Wolinsky, S. M., Letvin, N. L. (2002) Eventual AIDS vaccine failure in a rhesus monkey by viral escape from cytotoxic T lymphocytes. *Nature* **415**, 335-339.
- Barouch, D. H., Santra, S., Schmitz, J. E., Kuroda, M. J., Fu, T. M., Wagner, W., Bilska, M., Craiu, A., Zheng, X. X., Krivulka, G. R., Beaudry, K., Lifton, M. A., Nickerson, C. E., Trigona, W. L., Punt, K., Freed, D. C., Guan, L., Dubey, S., Casimiro, D., Simon, A., Davies, M. E., Chastain, M., Strom, T. B., Gelman, R. S., Montefiori, D. C., Lewis, M. G., Letvin, N. L. (2000) Control of viraemia and prevention of clinical AIDS in rhesus monkeys by cytokine-augmented DNA vaccination. *Science* **290**, 486-492.
- Borrow, P., Lewicki, H., Hahn, B. E., Shaw, G. M., Oldstone, M. B. (1994) Virus-specific CD8+ CTL activity associated with control of viraemia in primary HIV-1 infection. *J. Virol.* **68**, 6103-6110.
- Burton, D. R., Desrosiers, R. C., Doms, R. W., Feinberg, M. B., Gallo, R. C., Hahn, B., Hoxie, J. A., Hunter, E., Korber, B., Landay, A., Lederman, M. M., Lieberman, J., McCune, J. M., Moore, J. P., Nathanson, N., Picker, L., Richman, D., Rinaldo, C., Stevenson, M., Watkins, D. I., Wolinsky, S. M., Zack, J. A. (2004a) Public health. A sound rationale needed for phase III HIV-1 vaccine trials. *Science* **303**, 316.
- Burton, D. R., Desrosiers, R. C., Doms, R. W., Koff, W. C., Kwong, P. D., Moore, J. P., Nabel, G. J., Sodroski, J., Wilson, I. A., Wyatt, R. T. (2004b) HIV vaccine design and the neutralizing antibody problem. *Nat. Immunol.* **5**, 233-236.
- Buseyne, F., Burgard, M., Teglas, J. P., Bui, E., Rouzioux, C., Mayaux, M. J., Blanche, S., Riviere, Y. (1998) Early HIV-specific cytotoxic T lymphocytes and disease progression in children born to HIV-infected mothers. *AIDS Res. Hum. Retroviruses* **14**, 1435-1444.
- Buseyne, F., Le Chenadec, J., Corre, B., Porrot, F., Burgard, M., Rouzioux, C., Blanche, S., Mayaux, M. J., Riviere, Y. (2002) Inverse correlation between memory Gag-specific cytotoxic T lymphocytes and viral replication in human immunodeficiency virus-infected children. *J. Infect. Dis.* **186**, 1589-1596.
- Cafaro, A., Caputo, A., Fracasso, C., Maggiorella, M. T., Goletti, D., Baroncelli, S., Pace, M., Sernicola, L., Koanga-Mogtomo, M. L., Betti, M., Borsetti, A., Belli, R., Akerblom, L., Corrias, F., Butto, S., Heeney, J., Verani, P., Titti, F., Ensoli, B. (1999) Control of SHIV-89.6P infection of cynomolgus monkeys by HIV-1 Tat protein vaccine. *Nat. Med.* **5**, 643-650.
- Carrington, M., Nelson, G. W., Martin, M. P., Kissner, T., Vlahov, D., Goedert, J. J., Kaslow, R., Buchbinder, S., Hoots, K., O'Brien, S. J. (1999) HLA and HIV-1: heterozygote advantage and B\*35-Cw\*04 disadvantage. *Science* **283**, 1748-1752.
- Champagne, P., Ogg, G. S., King, A. S., Knabenhans, C., Ellefsen, K., Nobile, M., Appay, V., Rizzardì, G. P., Fleury, S., Lipp, M., Foster, R., Rowland-Jones, S., Sekaly, R. P.,

- McMichael, A. J., Pantaleo, G. (2001) Skewed maturation of memory HIV-specific CD8 T lymphocytes. *Nature* **410**, 106-111.
- Cheyrier, R., Langlade-Demoyen, P., Marescot, M. R., Blanche, S., Blondin, G., Wain-Hobson, S., Griscelli, C., Vilmer, E., Plata, F. (1992) Cytotoxic T lymphocyte responses in the peripheral blood of children born to human immunodeficiency virus-1-infected mothers. *Eur. J. Immunol.* **22**, 2211-2217.
- Datta, P., Embree, J. E., Kreiss, J. K., Ndinya-Achola, J. O., Braddick, M., Temmerman, M., Nagelkerke, N. J., Maitha, G., Holmes, K. K., Piot, P., et al. (1994) Mother-to-child transmission of human immunodeficiency virus type 1: report from the Nairobi Study. *J. Infect. Dis.* **170**, 1134-1140.
- De Maria, A., Cirillo, C., Moretta, L. (1994) Occurrence of human immunodeficiency virus type 1 (HIV-1)-specific cytolytic T cell activity in apparently uninfected children born to HIV-1-infected mothers. *J. Infect. Dis.* **170**, 1296-1299.
- Ekpini, E. R., Wiktor, S. Z., Satten, G. A., Adjorlolo-Johnson, G. T., Sibailly, T. S., Ou, C. Y., Karon, J. M., Brattegaard, K., Whitaker, J. P., Gnaore, E., De Cock, K. M., Greenberg, A. E. (1997) Late postnatal mother-to-child transmission of HIV-1 in Abidjan, Cote d'Ivoire. *Lancet* **349**, 1054-1059.
- Ferrantelli, F., Ruprecht, R. M. (2002) Neutralizing antibodies against HIV – back in the major leagues? *Curr. Opin. Immunol.* **14**, 495-502.
- Friedrich, T. C., Dodds, E. J., Yant, L. J., Vojnov, L., Rudersdorf, R., Cullen, C., Evans, D. T., Desrosiers, R. C., Mothe, B. R., Sidney, J., Sette, A., Kunstman, K., Wolinsky, S., Piatak, M., Lifson, J., Hughes, A. L., Wilson, N., O'Connor, D. H., Watkins, D. I. (2004) Reversion of CTL escape-variant immunodeficiency viruses in vivo. *Nat. Med.* **10**, 275-281.
- Gallimore, A., Cranage, M., Cook, N., Almond, N., Bootman, J., Rud, R., Silvera, P., Dennis, M., Corcoran, T., Stott, J., McMichael, A., Gotch, F. (1995) Early suppression of SIV replication by CD8+ nef-specific cytotoxic T cells in vaccinated animals. *Nat. Med.* **1**, 1167-1173.
- Gibbons, J., Cory, J. M., Hewlett, I. K., Epstein, J. S., Eyster, M. E. (1990) Silent infections with human immunodeficiency virus type 1 are highly unlikely in multitransfused seronegative hemophiliacs. *Blood* **76**, 1924-1926.
- Goh, W. C., Markee, J., Akridge, R. E., Meldorf, M., Musey, L., Karchmer, T., Krone, M., Collier, A., Corey, L., Emerman, M., McElrath, M. J. (1999) Protection against human immunodeficiency virus type 1 infection in persons with repeated exposure: evidence for T cell immunity in the absence of inherited CCR5 coreceptor defects. *J. Infect. Dis.* **179**, 548-557.
- Goulder, P. J., Pasquier, C., Holmes, E. C., Liang, B., Tang, Y., Izopet, J., Saune, K., Rosenberg, E. S., Burchett, S. K., McIntosh, K., Barnardo, M., Bunce, M., Walker, B. D., Brander, C., Phillips, R. E. (2001) Mother-to-child transmission of HIV infection and CTL escape through HLA-A2-SLYNTVATL epitope sequence variation. *Immunol. Lett.* **79**, 109-116.
- Goulder, P. J. R., Phillips, R. E., Colbert, R. A., McAdam, S., Ogg, G., Nowak, M. A., Giangrande, P., Luzzi, G., Morgan, B., Edwards, A., McMichael, A. J., Rowland-Jones, S. (1997) Late escape from an immunodominant cytotoxic T-lymphocyte response associated with progression to AIDS. *Nat. Med.* **3**, 212-217.
- Hanke, T. (2003) Development of prophylactic AIDS vaccines: the current state of affairs. *Curr. Opin. Mol. Ther.* **5**, 25-33.
- Harrer, T., Harrer, E., Kalams, S. A., Elbeik, T., Staprans, S. I., Feinberg, M. B., Cao, Y., Ho, D. D., Yilma, T., Caliando, A. M., Johnson, R. P., Buchbinder, S. P., Walker, B. D. (1996) Strong cytotoxic T cell and weak neutralizing antibody responses in a subset of persons with stable nonprogressing HIV type 1 infection. *AIDS Res. Hum. Retroviruses* **12**, 585-592.
- Jin, X., Bauer, D. E., Tuttleton, S. E., Lewin, S., Gettie, A., Blanchard, J., Irwin, C. E., Safrit, J. T., Mittler, J., Weinberger, L., Kostrikis, L. G., Zhang, L., Perelson, A. S., Ho, D. D. (1999) Dramatic rise in plasma viraemia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. *J. Exp. Med.* **189**, 991-998.
- Kaslow, R. A., Rivers, C., Tang, J., Bender, T. J., Goepfert, P. A., El Habib, R., Weinhold, K., Mulligan, M. J., Group, N. A. V. E. (2001) Polymorphisms in HLA class I gene associated with both favourable prognosis of human immunodeficiency virus (HIV) type 1 infection and positive cytotoxic T lymphocytes responses to ALVAC-HIV recombinant canarypox vaccines. *J. Virol.* **75**, 8681-8689.
- Kaul, R., Plummer, F. A., Kimani, J., Dong, T., Kiama, P., Rostron, T., Njagi, E., MacDonald, K. S., Bwayo, J. J., McMichael, A. J., Rowland-Jones, S. L. (2000) HIV-1-specific mucosal CD8+ lymphocyte responses in the cervix of HIV-1-resistant prostitutes in Nairobi. *J. Immunol.* **164**, 1602-1611.
- Kaul, R., Rowland-Jones, S. L., Kimani, J., Dong, T., Yang, H. B., Kiama, P., Rostron, T., Njagi, E., Bwayo, J. J., MacDonald, K. S., McMichael, A. J., Plummer, F. A. (2001) Late seroconversion in HIV-resistant Nairobi prostitutes despite pre-existing HIV-specific CD8+ responses. *J. Clin. Invest.* **107**, 341-349.
- Kelleher, A. D., Long, C., Holmes, E. C., Allen, R. L., Wilson, J., Conlon, C., Workman, C., Shaunak, S., Olson, K., Goulder, P., Brander, C., Ogg, G., Sullivan, J. S., Dyer, W., Jones, I., McMichael, A. J., Rowland-Jones, S., Phillips, R. E. (2001) Clustered mutations in HIV-1 gag are consistently required for escape from HLA-B27-restricted cytotoxic T lymphocyte responses. *J. Exp. Med.* **193**, 375-386.
- Kent, S. J., Woodward, A., Zhao, A. (1997) Human immunodeficiency virus type 1 (HIV-1)-specific T cell responses correlate with the control of acute HIV-1 infection in macaques. *J. Infect. Dis.* **176**, 1188-1197.
- Klenerman, P., Wu, Y., Phillips, R. (2002) HIV: current opinion in escapology. *Curr. Opin. Microbiol.* **5**, 408-413.
- Koup, R. A., Safrit, J. T., Cao, Y., Andrews, C. A., McLoed, G., Borkowsky, W., Farthing, C., Ho, D. D. (1994) Temporal association of cellular immune responses with the initial control of viraemia in primary human immunodeficiency virus type 1 syndrome. *J. Virol.* **68**, 4650-4655.
- Leslie, A. J., Pfafferott, K. J., Chetty, P., Draenert, R., Addo, M. M., Feeney, M., Tang, Y., Holmes, E. C., Allen, T., Prado, J. G., Altfeld, M., Brander, C., Dixon, C., Ramduth, D., Jeena, P., Thomas, S. A., John, A. S., Roach, T. A., Kupfer, B., Luzzi, G., Edwards, A., Taylor, G., Lyall, H., Tudor-Williams, G., Novelli, V., Martinez-Picado, J., Kiepiela, P., Walker, B. D., Goulder, P. J. (2004) HIV evolution: CTL escape mutation and reversion after transmission. *Nat. Med.* **10**, 282-289.
- Luzuriaga, K., Holmes, D., Hereema, A., Wong, J., Panicali, D. L., Sullivan, J. L. (1995) HIV-1-specific cytotoxic T

- lymphocyte responses in the first year of life. *J. Immunol.* **154**, 433-443.
- Luzuriaga, K., Sullivan, J. L. (2002) Pediatric HIV-1 infection: advances and remaining challenges. *AIDS Rev.* **4**, 21-26.
- Makori, N., Tarantal, A. F., Lu, F. X., Rourke, T., Marthas, M. L., McChesney, M. B., Hendrickx, A. G., Miller, C. J. (2003) Functional and morphological development of lymphoid tissues and immune regulatory and effector function in rhesus monkeys: cytokine-secreting cells, immunoglobulin-secreting cells, and CD5(+) B-1 cells appear early in fetal development. *Clin. Diagn. Lab. Immunol.* **10**, 140-153.
- Marchant, A., Appay, V., Van Der Sande, M., Dulphy, N., Liesnard, C., Kidd, M., Kaye, S., Ojuola, O., Gillespie, G. M., Vargas Cuero, A. L., Cerundolo, V., Callan, M., McAdam, K. P., Rowland-Jones, S. L., Donner, C., McMichael, A. J., Whittle, H. (2003) Mature CD8(+) T lymphocyte response to viral infection during fetal life. *J. Clin. Invest.* **111**, 1747-1755.
- Marlink, R., Kanki, P., Thior, I., Travers, K., Eisen, G., Siby, T., Traore, I., Hsieh, C. C., Dia, M. C., Gueye, E. H., et al. (1994) Reduced rate of disease development after HIV-2 infection as compared to HIV-1. *Science* **265**, 1587-1590.
- Matano, T., Shibata, R., Siemon, C., Connors, M., Lane, H. C., Martin, M. A. (1998) Administration of an anti-CD8 monoclonal antibody interferes with the clearance of chimeric simian/human immunodeficiency virus during primary infections of rhesus macaques. *J. Virol.* **72**, 164-169.
- Mazzoli, S., Trabattoni, D., Lo Caputo, S., Piconi, S., Ble, C., Meacci, F., Ruzzante, S., Salvi, A., Semplici, F., Longhi, R., Fusi, M. L., Tofani, N., Biasin, M., Villa, M. L., Mazzotta, F., Clerici, M. (1997) HIV-specific mucosal and cellular immunity in HIV-seronegative partners of HIV-seropositive individuals. *Nat. Med.* **3**, 1250-1257.
- Mbori-Ngacha, D., Nduati, R., John, G., Reilly, M., Richardson, B., Mwatha, A., Ndinya-Achola, J., Bwayo, J., Kreiss, J. (2001) Morbidity and mortality in breastfed and formula-fed infants of HIV-1-infected women: A randomized clinical trial. *JAMA* **286**, 2413-2420.
- McMichael, A. (1998) T cell responses and viral escape. *Cell* **93**, 673-676.
- Mo, H., Monard, S., Pollack, H., Ip, J., Rochford, G., Wu, L., Hoxie, J., Borkowsky, W., Ho, D. D., Moore, J. P. (1998) Expression patterns of the HIV type 1 coreceptors CCR5 and CXCR4 on CD4+ T cells and monocytes from cord and adult blood. *AIDS Res. Hum. Retroviruses* **14**, 607-617.
- Moore, C. B., John, M., James, I. R., Christiansen, F. T., Witt, C. S., Mallal, S. A. (2002) Evidence of HIV-1 adaptation to HLA-restricted immune responses at a population level. *Science* **296**, 1439-1443.
- O'Connor, D. H., Allen, T. M., Watkins, D. I. (2002) Cytotoxic T-lymphocyte escape monitoring in simian immunodeficiency virus vaccine challenge studies. *DNA Cell Biol.* **21**, 659-664.
- Ogg, G. S., Jin, X., Bonhoeffer, S., Dunbar, P. R., Nowak, M. A., Monard, S., Segal, J. P., Cao, Y., Rowland-Jones, S. L., Cerundolo, V., Hurley, A., Markowitz, M., Ho, D. D., Nixon, D. F., McMichael, A. J. (1998) Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. *Science* **279**, 2103-2106.
- Osterhaus, A. D., van Baalen, C. A., Gruters, R. A., Schutten, M., Siebelink, C. H., Hulskotte, E. G., Tijhaar, E. J., Randall, R. E., van Amerongen, G., Fleuchaus, A., Erfle, V., Sutter, G. (1999) Vaccination with Rev and Tat against AIDS. *Vaccine* **17**, 2713-2714.
- Otsyula, M. G., Miller, C. J., Tarantal, A. F., Marthas, M. L., Greene, T. P., Collins, J. R., van Rompay, K. K., McChesney, M. B. (1996) Fetal or neonatal infection with attenuated simian immunodeficiency virus results in protective immunity against oral challenge with pathogenic SIVmac251. *Virology* **222**, 275-278.
- Pikora, C. A., Sullivan, J. L., Panicali, D., Luzuriaga, K. (1997) Early HIV-1 envelope-specific cytotoxic T lymphocyte responses in vertically infected infants. *J. Exp. Med.* **185**, 1153-1161.
- Pinto, L. A., Sullivan, J., Berzofsky, J. A., Clerici, M., Kessler, H. A., Landay, A. L., Shearer, G. M. (1995) Envelope-specific cytotoxic T lymphocytes in HIV seronegative health care workers occupationally exposed to HIV-contaminated body fluids. *J. Clin. Invest.* **96**, 867-876.
- Price, D. A., Sewell, A. K., Dong, T., Tan, R., Goulder, P. J., Rowland-Jones, S. L., Phillips, R. E. (1998) Antigen-specific release of beta-chemokines by anti-HIV-1 cytotoxic T lymphocytes. *Curr. Biol.* **8**, 355-358.
- Richardson, B. A., John-Stewart, G. C., Hughes, J. P., Nduati, R., Mbori-Ngacha, D., Overbaugh, J., Kreiss, J. K. (2003a) Breast-milk infectivity in human immunodeficiency virus type 1-infected mothers. *J. Infect. Dis.* **187**, 736-740.
- Richardson, B. A., Mbori-Ngacha, D., Lavreys, L., John-Stewart, G. C., Nduati, R., Panteleeff, D. D., Emery, S., Kreiss, J. K., Overbaugh, J. (2003b) Comparison of human immunodeficiency virus type 1 viral loads in Kenyan women, men, and infants during primary and early infection. *J. Virol.* **77**, 7120-7123.
- Rousseau, C. M., Nduati, R. W., Richardson, B. A., Steele, M. S., John-Stewart, G. C., Mbori-Ngacha, D. A., Kreiss, J. K., Overbaugh, J. (2003) Longitudinal analysis of human immunodeficiency virus type 1 RNA in breast milk and of its relationship to infant infection and maternal disease. *J. Infect. Dis.* **187**, 741-747.
- Rowland-Jones, S., Sutton, J., Ariyoshi, K., Dong, T., Gotch, F., McAdam, S., Whitby, D., Sabally, S., Gallimore, A., Corrah, T., Takiguchi, M., Schultz, T., McMichael, A., Whittle, H. (1995) HIV-specific cytotoxic T-cells in HIV-exposed but uninfected Gambian women. *Nat. Med.* **1**, 59-64.
- Rowland-Jones, S. L., Dong, T., Fowke, K. R., Kimani, J., Krausa, P., Newell, H., Blanchard, T., Ariyoshi, K., Oyugi, J., Ngugi, E., Bwayo, J. J., MacDonald, K. S., McMichael, A. J., Plummer, F. A. (1998) Cytotoxic T cell responses to multiple conserved epitopes in HIV-resistant prostitutes in Nairobi. *J. Clin. Inv.* **102**, 1758-1765.
- Rowland-Jones, S. L., McMichael, A. (1995) Immune responses in HIV-exposed seronegatives: have they repelled the virus? *Curr. Opin. Immunol.* **7**, 448-455.
- Rowland-Jones, S. L., Nixon, D. F., Aldhous, M. C., Gotch, F., Ariyoshi, K., Hallam, N., Kroll, J. S., Froebel, K., McMichael, A. (1993) HIV-specific cytotoxic T-cell activity in an HIV-exposed but uninfected infant. *Lancet* **341**, 860-861.
- Safrit, J. T., Ruprecht, R., Ferrantelli, F., Xu, W., Kitabwalla, M., Van Rompay, K., Marthas, M., Haigwood, N., Mascola, J. R., Luzuriaga, K., Jones, S. A., Mathieson, B. J., Newell, M. L. (2004) Immunoprophylaxis to prevent mother-to-child transmission of HIV-1. *J. Acquir. Immune Defic. Syndr.* **35**, 169-177.

- Schmitz, J. E., Kuroda, M. J., Sasseville, V. G., Simon, M. A., Lifton, M. A., Racz, P., Tenner-Racz, K., Dalesandro, M., Scallon, B. J., Ghayeb, J., Forman, M. A., Montefiori, D. C., Rieber, E. P., Letvin, N. L., Reinmann, K. A. (1999) Control of viraemia in simian immunodeficiency virus infection by CD8+ lymphocytes. *Science* **283**, 857-860.
- Scott, Z. A., Chadwick, E. G., Gibson, L. L., Catalina, M. D., McManus, M. M., Yogev, R., Palumbo, P., Sullivan, J. L., Britto, P., Gay, H., Luzuriaga, K. (2001) Infrequent detection of HIV-1-specific, but not cytomegalovirus-specific, CD8(+) T cell responses in young HIV-1-infected infants. *J. Immunol.* **167**, 7134-7140.
- Spiegel, H. M., Chandwani, R., Sheehy, M. E., Dobroszycki, J., Fennelly, G., Wiznia, A., Radding, J., Rigaud, M., Pollack, H., Borkowsky, W., Rosenberg, M., Nixon, D. F. (2000) The impact of early initiation of highly active anti-retroviral therapy on the human immunodeficiency virus type 1-specific CD8 T cell response in children. *J. Infect. Dis.* **182**, 88-95.
- Stranford, S. A., Skurnick, J., Louria, D., Osmond, D., Chang, S. Y., Sninsky, J., Ferrari, G., Weinhold, K., Lindquist, C., Levy, J. A. (1999) Lack of infection in HIV-exposed individuals is associated with a strong CD8(+) cell noncytotoxic anti-HIV response. *Proc. Natl. Acad. Sci. USA* **96**, 1030-1035.
- UNAIDS (2001). AIDS Epidemic Update: December 2001. Geneva, UNAIDS/WHO.
- Van de Perre, P., Simonon, A., Msellati, P., Hitimana, D. G., Vaira, D., Bazubagira, A., Van Goethem, C., Stevens, A. M., Karita, E., Sondag-Thull, D., et al. (1991) Postnatal transmission of human immunodeficiency virus type 1 from mother to infant. A prospective cohort study in Kigali, Rwanda. *N. Engl. J. Med.* **325**, 593-598.
- Van Rompay, K. K., Greenier, J. L., Cole, K. S., Earl, P., Moss, B., Steckbeck, J. D., Pahar, B., Rourke, T., Montelaro, R. C., Canfield, D. R., Tarara, R. P., Miller, C., McChesney, M. B., Marthas, M. L. (2003) Immunization of newborn rhesus macaques with simian immunodeficiency virus (SIV) vaccines prolongs survival after oral challenge with virulent SIVmac251. *J. Virol.* **77**, 179-190.
- Wagner, L., Yang, O. O., Garcia-Zepeda, E. A., Ge, Y., Kalams, S. A., Walker, B. D., Pasternack, M. S., Luster, A. D. (1998) Beta-chemokines are released from HIV-1-specific cytolytic T cell granules complexed to proteoglycans. *Nature* **391**, 908-911.
- Walker, B. D., Chakrabarti, S., Moss, B., Paradis, T. J., Flynn, T., Durno, A. G., Blumberg, R. S., Kaplan, J. C., Hirsch, M. S., Schooley, R. T. (1987) HIV-specific cytotoxic T lymphocytes in seropositive individuals. *Nature* **328**, 345-348.
- Wasik, T. J., Jagodzinski, P. P., Hyjek, E. M., Wustner, J., Trinchieri, G., Lischner, H. W., Kozbor, D. (1997) Diminished HIV-specific CTL activity is associated with lower type 1 and enhanced type 2 responses to HIV-specific peptides during perinatal HIV infection. *J. Immunol.* **158**, 6029-6036.
- Wasik, T. J., Wierzbicki, A., Whiteman, V. E., Trinchieri, G., Lischner, H. W., Kozbor, D. (2000) Association between HIV-specific T helper responses and CTL activities in pediatric AIDS. *Eur. J. Immunol.* **30**, 117-127.
- Wilson, C. C., Brown, R. C., Korber, B. T., Wilkes, B. M., Ruhl, D. J., Sakamoto, D., Kunstman, K., Luzuriaga, K., Hanson, I. C., Widmayer, S. M., Wiznia, A., Clapp, S., Ammann, A. J., Koup, R. A., Wolinsky, S. M., Walker, B. D. (1999) Frequent detection of escape from cytotoxic T-lymphocyte recognition in perinatal human immunodeficiency virus (HIV) type 1 transmission: the Ariel Project for the prevention of transmission of HIV from mother to infant. *J. Virol.* **73**, 3975-3985.
- Yusim, K., Kesmir, C., Gaschen, B., Addo, M. M., Altfeld, M., Brunak, S., Chigaev, A., Detours, V., Korber, B. T. (2002) Clustering patterns of cytotoxic T-lymphocyte epitopes in human immunodeficiency virus type 1 (HIV-1) proteins reveal imprints of immune evasion on HIV-1 global variation. *J. Virol.* **76**, 8757-8768.
- Zajac, A. J., Blattman, J. N., Murali-Krishna, K., Sourdive, D. J., Suresh, M., Altman, J. D., Ahmed, R. (1998) Viral immune evasion due to persistence of activated T cells without effector function. *J. Exp. Med.* **188**, 2205-2213.
- Zhang, C., Cui, Y., Houston, S., Chang, L. J. (1996) Protective immunity to HIV-1 in SCID/beige mice reconstituted with peripheral blood lymphocytes of exposed but uninfected individuals. *Proc. Natl. Acad. Sci. USA* **93**, 14720-14725.
- Zhu, T., Corey, L., Hwangbo, Y., Lee, J. M., Learn, G. H., Mullins, J. I., McElrath, M. J. (2003) Persistence of extraordinarily low levels of genetically homogeneous human immunodeficiency virus type 1 in exposed seronegative individuals. *J. Virol.* **77**, 6108-6116.
- Ziegner, U., Campbell, D., Weinhold, K., Frank, I., Rutstein, R., Starr, S. E. (1999) Deficient antibody-dependent cellular cytotoxicity against human immunodeficiency virus (HIV)-expressing target cells in perinatal HIV infection. *Clin. Diagn. Lab. Immunol.* **6**, 718-724.