



Bridging the Gap: Human Diploid Cell Strains and the Origin of AIDS

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Recent descriptions of the first human and chimpanzee cases of human immunodeficiency virus type 1 (HIV-1)-related retroviral infections dating from 1959 have stirred interest in the origin of AIDS. Although the theory of a chimpanzee origin of HIV-1 with cross-species transfer to man has now gained popularity, a more likely scenario is that chimps and humans were infected by an HIV-1 precursor virus derived from a contaminated poliovaccine. The reason for the rapidity and ease of cross-species transfer of this precursor virus has not been elucidated. We hypothesize that the poliovaccine was passaged in a human diploid cell strain. This simple manipulation allowed the retrovirus to adapt to human tissues and may have spawned the AIDS pandemic.

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Introduction

Although for many years it was assumed that human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) diverged from a precursor simian immunodeficiency virus (SIV) thousands of years ago, clinical and virologic observations suggest a more recent split between the simian and human retroviruses. The clinical evidence is based on the fact that no HIV infection of any kind has ever been documented prior to 1959 (Elswood & Stricker, 1994; Myers, 1994). The virologic evidence derives from studies showing that the first isolates of an HIV-1 like virus in humans and chimpanzees are consistent with SIV/HIV divergence in the 1950s (Gao *et al.*, 1999; Zhu *et al.*, 1999). It is likely that casual or random human infection with SIV produced the low-virulence HIV-2 strain in West Africa (Essex, 1994), although a limited poliovaccine trial may

have contributed to this zoonosis (Hooper, 1999). In contrast, it appears that the more virulent HIV-1 strains entered the human population in a systematic fashion consistent with extensive exposure to a contaminated vaccine. The following report focuses on two such events.

The theory that the AIDS pandemic evolved from a contaminated poliovaccine has been controversial (Goldberg, 1992; Curtis 1992; Elswood & Stricker, 1993; Hooper, 1999). A problem with this hypothesis is that an AIDS precursor virus would have had to jump the species barrier and adapt to human cells in a relatively short period of time. This event would require both a readily adaptable virus and a susceptible host. It is likely that HIV-1 originated from poliovirus seed lots passaged in primary monkey kidney cells infected with SIV. We now know that primary monkey kidney cells used to prepare most live poliovaccines were reservoirs for SIV and other retroviruses (Feldman *et al.*, 1975; Essex & Kanki, 1988; Neumann-Haeflin *et al.*, 1993; Linial, 1999). Furthermore, recent evidence

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confirms that a variety of monkey kidney cells (including chimpanzee kidney cells) were used in the early days of oral poliovaccine production (Hooper, 1999).

We suggest that there were two epidemics involving contaminated poliovaccines. The epidemics on different continents were facilitated by the method used to produce the vaccines and by the populations inoculated with the resultant vaccine products. Based on this hypothesis, it is probable that the African heterosexual HIV-1 non-B subtypes had a somewhat different origin from the U.S. homosexual HIV-1 subtype B. As outlined below, the experimental poliovaccine given to infants and children in Central Africa may have been part of a field trial involving a new vaccine production method using human cells (Goldberg, 1992; Hayflick, 1992). In the United States, well-intentioned misuse of a retrovirus-containing poliovaccine in homosexual adults and subsequent development of a hepatitis B vaccine could have unwittingly spread HIV into the homosexual population (Kyle, 1992).

Human Diploid Cell Strains

In the 1950s, poliovaccine production utilized fresh monkey kidney (MK) explants to attenuate the poliovirus (Elswood & Stricker, 1994). Among vaccine researchers, there was great concern about contamination of these primary MK cells with known monkey viruses such as cytomegalovirus and simian virus 40 (SV40) (Shah & Nathanson, 1976; Kyle, 1992). Hayflick *et al.* (1962) explain: "The utilization of primary MK cells for human vaccine production has certain inherent disadvantages: (a) high latent virus content, (b) survival of SV40 in attenuated and formalinized poliovirus vaccine made in these cells, and (c) production of tumors by extracts of primary MK cells at the site of inoculation of hamsters, etc. To avoid these and other problems with the production of human virus vaccines in MK, we have attempted to demonstrate the efficacy of an attenuated poliovirus vaccine produced in an entirely different *in vitro* system. This system involves the use of human fetal diploid cell strains as a substrate for virus multiplication".

The use of human diploid cell strains was a major advance in the preparation of vaccines because it eliminated contamination by "known" simian viruses common in MK cells. According to Hayflick *et al.* (1962), "Poliovirus seed pools previously grown in monkey kidney can, apparently, be freed of simian viruses by serial plaque purification on human diploid cells, and live attenuated or killed virulent vaccine prepared in human diploid cells". Use of "normal" human diploid cell strains also avoided the unknown risks of passaging poliovirus in malignant human cell lines such as HeLa (Hayflick, 1963, 1964).

With the encouragement and support of the National Institutes of Health, Hayflick and his co-workers at the Wistar Institute in Philadelphia were experimenting with human diploid cell strains in the mid-1950s, resulting in their WI-38 exemplar strain that was described in 1961 (Hayflick & Moorhead, 1961). Development of the earlier strains (e.g. WI-1 to WI-37) apparently started five years earlier (Hayflick, 1998). Hayflick explained this in 1961: ". . . Dr Moorhead and I have been active in this area for some time and have at present accumulated no less than 50 diploid cell strains, predominantly from human fetal skin and muscle, lung, liver, and kidney" (Hayflick, 1961). Thus, by the late 1950s, human diploid cell strains were poised to revolutionize vaccine production throughout the world.

Epidemic # 1: HIV-1 Subtype non-B [HIV-1 (non-B)]

In 1957–1960, a mass poliovaccine immunization program was undertaken in the Belgian Congo (Curtis, 1992; Elswood & Stricker, 1994). Ultimately, more than 320 000 infants and children were given an oral poliovaccine derived from the CHAT strain developed at the Wistar Institute. The CHAT strain was passaged on monkey kidney cells and contained at least one "unidentified non-poliomyelitis virus" contaminant (Elswood & Stricker, 1993).

As concerns the poliovaccine used in the Belgian Congo, Hayflick (1992) states: "The seed virus used by me to produce the type 1 poliovirus vaccine was the CHAT strain that had been grown previously in primary monkey kidney

cells. . . . Before production of the vaccine, the type 1 CHAT seed virus was terminally diluted and triple plaque purified in high population doubling level (PDL) WI-1. This material was then used to produce the seed virus in WI-1 and PDL 22. Although triple plaque purification is not a guarantee that a putative unwanted virus was removed, the possibility that HIV-1 or any other virus may have remained is vanishingly small" (Hayflick, 1992).

WI-1 is a human fetal lung cell strain (Hayflick & Moorhead, 1961), and similar fetal lung cells are capable of supporting HIV-1 infection with reduced cytopathogenicity (Dolei *et al.*, 1994). Furthermore, human fetal fibroblast cell lines express the retroviral receptors CD4 and CCR-5, and these cells are susceptible to productive infection with SIVsm, SIVmac, SIVcpz and HIV-1 (Werner *et al.*, 1990; Chen *et al.*, 1997; Fazeley *et al.*, 1997; Rottman *et al.*, 1997). Since retroviruses integrate into genomic DNA, proliferation of human fetal lung cells would result in productive infection of daughter cells. Thus, retroviral infection of WI-1 during "triple plaque purification" could result in dynamic viral diversification and adaptation of the monkey-derived virus to human cells.

Prior to the mass vaccination program in the Belgian Congo, a "gang-caged" colony of as many as 400 chimpanzees was established in Camp Lindi at Stanleyville (now Kisangani, Zaire) to evaluate attenuated strains of poliovirus in chimpanzees (Curtis, 1992; Courtois *et al.*, 1958; Hooper, 1999). The chimps came from the area around Ponthierville in the eastern Congo (now Ubundu, Zaire), and they were primarily *Pan troglodytes* species (Vangroenweghe, 1997). Courtois *et al.* (1958) explain: "... caretakers of the animal colony were vaccinated with attenuated virus in order to protect them against possible exposure to the virulent poliomyelitis used for challenge of vaccinated chimpanzees."

If HIV-1 actually originated from SIV infection of chimpanzees (Gao *et al.*, 1999), it is conceivable that the poliovaccine being prepared at Camp Lindi was contaminated by SIV-infected chimpanzees or caretakers at the test colony. Alternatively, the chimps and their caretakers may have been "ground zero" for the first live primate exposure to HIV-1 from the contaminated poliovaccine that was passed on WI-1.

As part of the Congo vaccine trial, the Leopoldville vaccination program involved 154 000 African children aged 6 months to 2 years, who were inoculated by directly spraying the poliovaccine into their respiratory tracts (Plotkin *et al.*, 1961). If the poliovaccine was contaminated, infection could have resulted from exposure of tissue and alveolar macrophages and/or epithelial cells in the respiratory tract to a human-adapted SIV precursor of HIV-1 (Rich *et al.*, 1992; Mellert *et al.*, 1990; Meltzer & Gendelman, 1992; Stahl-Hennig *et al.*, 1999). In the early 1970s, when these children reached puberty, engaged in sex, and had children, Epidemic #1 could have resulted. Migration to and from Central Africa could have resulted in the worldwide spread of HIV-1 (non-B). The diversity of the non-B HIV-1 clades may be related to viral incubation in the African population prior to worldwide dissemination (Myers, 1994; Jonassen *et al.*, 1997).

In the early 1980s, data were obtained that focused on medical and immune system abnormalities in Central Africa, particularly non-endemic Kaposi's sarcoma (KS) (Nahmias *et al.*, 1986; Clumeck *et al.*, 1984; Van de Perre *et al.*, 1984; Piot *et al.*, 1984). The highest incidence of KS was located in the area where the Congo poliovaccine program of 1957–1960 had taken place (Elswood & Stricker, 1994).

Epidemic #2: HIV-1 Subtype B (HIV-1B)

By the 1970s, human diploid cell strains had entered into vaccine production in the U.S. (Hayflick, 1998, 1992). WI-38 was used to produce poliovaccines from poliovirus seed lots prepared in African green monkey kidney cells, which are reservoirs of SIV (Essex & Kanki, 1988). Like its predecessor WI-1, WI-38 is a human fetal lung cell strain (Hayflick, 1985). As stated previously, similar fetal lung cells are capable of supporting HIV-1 infection with reduced cytopathogenicity (Dolei *et al.*, 1994), and human fetal fibroblast cell lines are susceptible to productive infection with SIV and HIV-1 (Werner *et al.*, 1990; Chen *et al.*, 1997). WI-38 was used in the production of the Sabin poliovaccine, and it is possible that the vaccine produced in the early 1970s was contaminated with a retrovirus (Kyle,

1992). Reverse transcriptase was only discovered in 1970 (Baltimore, 1970; Temin & Mizutani, 1970), and no screening test for retroviruses existed at that time.

In the 1970s, oral and genital herpes simplex virus infection was pandemic in sexually active homosexual men, and there was no effective treatment. In urban areas that were centers for homosexual activity, these afflicted individuals sought out alternative treatments for the painful effects of herpes simplex outbreaks. At that time, the Sabin poliovaccine was used to treat recurrent genital herpes simplex in homosexual men. Multiple poliovaccine doses were given monthly to hundreds of these patients by clinicians in New York and San Francisco (Kyle, 1992; Lincoln & Nordstrom, 1976; Tager, 1974). Many homosexual men were relatively immunocompromised by sexually transmitted diseases, antibiotics, recreational drugs and intestinal parasites (Letvin, 1998). Exposure of these men to retrovirally contaminated poliovaccine in high doses would provide a gateway for HIV-1 infection and spread in the homosexual community (Smith, 1997; Moyer *et al.*, 1990).

Before AIDS, the major fear of homosexual men was hepatitis B virus (HBV) infection. To remedy this problem, an HBV vaccine was formulated during the mid-1970s using highly purified HBV surface antigen (HbsAg) particles derived from the plasma of chronic carriers of the virus (Szmunn *et al.*, 1980). These HBV carriers were New York City homosexual men attending bathhouses, venereal disease clinics, or other such "meeting" places (Chase, 1982). The highly purified HbsAg vaccine particles were treated with formaldehyde to kill any possible residual live virus (Szmunn *et al.*, 1980). However, infectious HIV-1 has been recovered from cells treated with formaldehyde (Aloisio & Nicholson, 1990).

Hepatitis B vaccine trials in high-risk HBV-negative homosexual men took place in the late 1970s and early 1980s. The first New York City participant was inoculated in November 1978, while the five-city trial (Los Angeles, San Francisco, St. Louis, Chicago, Denver), which was done in clinics for sexually transmitted diseases, began in March 1980 (Francis *et al.*, 1982; Szmunn *et al.*, 1980, 1981a). The HBV vaccine trial included two high-risk groups: dialysis patients

and homosexual men. The original vaccine protocol called for the use of HbsAg subtype "adw" derived from homosexual men for the vaccine given to homosexual men and HbsAg subtype "ayw" derived from dialysis patients for the vaccine given to dialysis patients (Szmunn, 1979). According to the subsequent published studies, however, a formaldehyde-treated vaccine containing HbsAg subtype "ad" was used in both the homosexual and dialysis patient trials, although the antigen donors were not identified (Szmunn *et al.*, 1981b, 1982; Stevens *et al.*, 1984). Subsequent HBV vaccines given to healthcare workers were treated with heat inactivation and pasteurization, which effectively eliminated the risk of retroviral contamination from plasma donors (Lelie *et al.*, 1987; Mauler *et al.*, 1987).

Samples from at least seven homosexual men were shown to be HIV-1 antibody-positive prior to entry into the New York City HBV vaccine trial (Stevens *et al.*, 1985). If some of the HBV-positive antigen donors were directly infected with HIV-1 or a precursor virus from the poliovaccine herpes treatment (or indirectly infected through sex with poliovaccine-treated individuals), the formaldehyde-treated HBV vaccine would have been contaminated. Anorectal and colon pathology (gay bowel syndrome) resulting in mucosal breakdown would have increased the risk and ease of HIV-1 transmission (Kazal *et al.*, 1976). If the injected HBV vaccine, given to high-risk and sexually active homosexuals residing in six major cities, was contaminated with HIV-1 or a precursor virus, it could explain the seemingly instantaneous and full-blown HIV-1 epidemic in the United States during the early 1980s (Table 1). Thus, Epidemic #2 could have resulted from the urban vaccination program, transforming a low-level infectious disease into a single-clade "starburst" as suggested by recently published AIDS statistics (Stine, 1998). Blood donations, intravenous drug use, travel and promiscuous sex could have caused the worldwide spread of HIV-1B.

Evolution of Poliovaccines

As outlined above, no safeguards against retroviral contamination of poliovaccines were available in the late 1950s in Africa or in the early

TABLE 1
New HIV infections and AIDS deaths in the
United States*

Year	New HIV infections	AIDS deaths
1977	19 000	—
1978	40 000	—
1979	60 000	—
1980	90 000	30 (before 1981)
1981	160 000	120
1982	190 000	446
1983	160 000	1473
1984	120 000	3447
1985	70 000	6853
1986	60 000	11 909
1987	50 000	16 065
1988	40 000	20 755
1989	40 000	27 351
1990	40 000	31 446
1991	40 000	35 989
1992	40 000	40 268
1993	40 000	42 949
1994	40 000	47 636
1995	40 000	49 351
1996	40 000	36 792
1997	40 000	21 222
1998	40 000	17 047

*Adapted from Stine (1998).

1970s in the U.S. In the mid-1970s, to prevent poliovaccine contamination by infectious agents from non-human primates, controlled monkey breeding colonies were established in the Caribbean basin (Lerche *et al.*, 1994). Subsequently, live oral poliovirus vaccine monopools produced in North America between 1976 and 1989 were tested by U.S. Food and Drug Administration researchers using the reverse transcriptase (RT) assay and polymerase chain reaction (PCR). None of the assays detected the presence of HIV-1/SIV, although some of the vaccine lots showed bands that were “faintly detectable in long-exposure autoradiographs” (Khan *et al.*, 1996). This study “was done to allay public fears and concerns regarding potential contamination of U.S.-licensed polio vaccine with HIV-1” (Khan *et al.*, 1997). Extrapolation of the data to all poliovaccine lots produced in previous years using “imported” monkey kidney cells, prior to screening with RT and PCR assays and before HIV/SIV was discovered, would require a leap of faith (Stricker & Goldberg, 1997).

WI-38 and the related human diploid cell strain MRC-5 continue to be used in the production of polio and other vaccines (Candal *et al.*, 1991). In addition, African green monkey kidney cell lines have been used for oral poliovaccine production in Europe since 1988, and tumor cell lines are now being considered for vaccine development (Vogel, 1999).

Conclusions

At least two different epidemics arose on different continents at different times: (1) heterosexuals in Africa infected with HIV-1 (non-B), and (2) homosexuals in the United States infected with HIV-1B. Statistically, such an event would be unlikely unless the gap between the epidemics was bridged. We now know that human diploid cell strains were used to produce poliovaccines, and that the cells are theoretically capable of supporting SIV and HIV-1 replication. We propose that human diploid cell strains were the vehicle that “bridged the gap”, resulting in SIV/HIV zoonosis from monkeys to humans. The purpose of using human diploid cell strains was to eliminate “known” simian virus contamination of vaccines. Even with the best of intentions, however, it would have been impossible to eliminate “unknown” simian retroviral contamination in the pre-AIDS era. This lesson becomes particularly relevant as we now contemplate AIDS vaccine trials using novel vaccine production strategies in developing and developed nations.

REFERENCES

- ALOISIO, C. H. & NICHOLSON, J. K. (1990). Recovery of infectious human immunodeficiency virus from cells treated with 1% paraformaldehyde. *J. Immunol. Methods* **128**, 281–285.
- BALTIMORE, D. (1970). Viral RNA-dependent DNA polymerase. *Nature* **226**, 1209–1211.
- CANDAL, F. J., GEORGE, V. G. & ADES, E. W. (1991). Possibilities of vaccine manufacture in human diploid cell strains with a serum replacement factor. *Biologicals* **19**, 213–218.
- CHASE, A. (1982). *Magic Shots*. New York: William Morrow & Company.
- CHEN, Z., ZHOU, P., HO, D. D., LANDAU, N. R. & MARX, P. A. (1997). Genetically divergent strains of simian immunodeficiency virus use CCR5 as a coreceptor for entry. *J. Virol.* **71**, 2705–2714.

- CLUMECK, N., SONNET, J., TAELEMAN, H., MASCART-LEMONE, F., DE BRUYERE, M., VANDEPERRE, P., DASNOY, J., MARCELIS, L., LAMY, M., JONAS, C., EYCKMANS, L., NOEL, H., VANHAEVERBEEK, M. & BUTZLER, J.-P. (1984). Acquired immunodeficiency syndrome in African patients. *N. Engl. J. Med.* **310**, 492-497.
- COURTOIS, G., FLACK, A., JERVIS, G. A. & KOPROWSKI, H. (1958). Preliminary report on mass vaccination of man with live attenuated poliomyelitis virus in the Belgian Congo and Ruanda-Urundi. *Br. Med. J.* **2**, 187-190.
- CURTIS, T. (1992). The origin of AIDS. *Rolling Stone* **626**, 54-59, 61, 106, 108.
- DOLEI, A., SERRA, C., ARCA, M. V., TILOCCA, F., RIVA, E., ANTONELLI, G., DIANZANI, F. & TONIOLO, A. (1994). Infectious virus with reduced cytopathogenicity resulting from persistent infection of normal lung fibroblasts by HIV type 1 strains. *AIDS Res. Hum. Retroviruses* **10**, 1089-1095.
- ELSWOOD, B. F. & STRICKER, R. B. (1993). Polio vaccines and the origin of AIDS. *Res. Virol.* **144**, 175-177.
- ELSWOOD, B. F. & STRICKER, R. B. (1994). Polio vaccines and the origin of AIDS. *Med. Hypotheses* **42**, 347-354.
- ESSEX, M. (1994). Simian immunodeficiency virus in people. *N. Engl. J. Med.* **330**, 209-210.
- ESSEX, M. & KANKI, P. (1988). The origin of the AIDS virus. *Sci. Am.* **259**, 64-71.
- FAZELEY, F., HU, J., THIRKILL, T. L. & DOUGLAS, G. C. (1997). Infection of primary human placental fibroblasts with HIV-1, HIV-2, and SIV. *Arch. Virol.* **142**, 2237-2248.
- FELDMAN, M. D., DUNNICK, N. R., BARRY, D. W. & PARKMAN, P. D. (1975). Isolation of foamy virus from rhesus, African green and cynomolgus monkey leukocytes. *J. Med. Primatol.* **4**, 287-295.
- FRANCIS, D. P., HALDER, S. C., THOMPSON, S. E. MAYNARD, J. E., OSTROW, D. G., ALTMAN, N., BRAFF, E. H. O'MALLEY, P., HAWKINS, D., JUDSON, F. N., PENLEY, K., NYLUND, T., CHRISTIE, G., MEYERS, F., MOORE, JR. J. N., GARDNER, A., DOTO, I. L., MILLER, J. H., REYNOLDS, G. H., MURPHY, B. L., SCHABLE, C. A., CLARK, B. T., CURRAN, J. W. & REDEKER, A. G. (1982). The prevention of hepatitis B with vaccine. Report of the centers for disease control multi-center efficacy trial among homosexual men. *Ann. Intern. Med.* **97**, 362-366.
- GAO, F., BAILES, E., ROBERTSON, D. L., CHEN, Y., RODENBURG, C. M., MICHAEL, S. F., CUMMINS, L. B., ARTHUR, L. O., PEETERS, M., SHAW, G. M., SHARP, P. M. & HAHN, B. H. (1999). Origin of HIV-1 in the chimpanzee *Pan troglodytes troglodytes*. *Nature* **397**, 436-441.
- GOLDBERG, B. (1992). Origin of AIDS. *Lancet* **339**, 1548.
- HAYFLICK, L. (1961). Comments. In: *Syvertsen Memorial Symposium: Analytic Cell Culture* (Stevenson, R. E., ed.), p. 82. Bethesda, MD: NCI.
- HAYFLICK, L. (1963). A comparison of primary monkey kidney, heteroploid cell lines, and human diploid cell strains for human virus vaccine preparation. *Amer. Rev. Respir. Dis.* **88**, 387-393.
- HAYFLICK, L. (1964). Advances in tissue culture methods important to viral disease problems. *Postgrad. Med.* **35**, 503-511.
- HAYFLICK, L. (1985). The cell biology of aging. *Clin. Geriatr. Med.* **1**, 15-27.
- HAYFLICK, L. (1992). Origin of HIV-1. *Lancet* **340**, 484-485.
- HAYFLICK, L. (1998). A novel technique for transforming the theft of mortal human cells into praiseworthy federal policy. *Exp. Gerontol.* **33**, 191-207.
- HAYFLICK, L. & MOORHEAD, P. S. (1961). The serial cultivation of human diploid cell strains. *Exp. Cell. Res.* **25**, 585-621.
- HAYFLICK, L., PLOTKIN, S. A., NORTON, T. W. & KOPROWSKI, H. (1962). Preparation of poliovirus vaccines in a human fetal diploid cell strain. *Am. J. Hyg.* **75**, 240-258.
- HOOPER, E. (1999). *The River: A Journey to the Source of HIV and AIDS*. New York: Little, Brown & Co.
- JONASSEN, T. O., STENE-JOHANSEN, K., BERG, E. S., HUNGNES, O., LINDBOE, C. F., FROLAND, S. S. & GRINDE, B. (1997). Sequence analysis of HIV-1 Group O from Norwegian patients infected in the 1960s. *Virology* **231**, 43-47.
- KAZAL, H. L., SOHN, N., CARRASCO, J. I., ROBILOTTI, J. G. & DELANEY, W. E. (1976). The gay bowel syndrome: clinicopathologic correlation in 260 cases. *Ann. Clin. Lab. Sci.* **6**, 184-192.
- KHAN, A. S., SHAHABUDDIN, M., BRYAN, T., JOSHI, B. H., LEE, S. & HEWLETT, I. K. (1990). Analysis of live, oral poliovirus vaccine monopools for human immunodeficiency virus type 1 and simian immunodeficiency virus. *J. Infect. Dis.* **174**, 1185-1190.
- KHAN, A. S., SHAHABUDDIN, M., BRYAN, T., JOSHI, B. H., LEE, S. & HEWLETT, I. K. (1997). Polio vaccines and retroviral contamination. *J. Infect. Dis.* **176**, 545-546.
- KYLE, W. S. (1992). Simian retroviruses, poliovaccine, and origin of AIDS. *Lancet* **339**, 600-601.
- LELIE, P. N., REESINK, H. W. & LUCAS, C. J. (1987). Inactivation of 12 viruses by heating steps applied during manufacture of a hepatitis B vaccine. *J. Med. Virol.* **23**, 297-301.
- LERCHE, N. W., YEE, J. L. & JENNINGS, M. B. (1994). Establishing specific retrovirus-free breeding colonies of macaques: an approach to primary screening and surveillance. *Lab. Anim. Sci.* **44**, 217-221.
- LETVIN, N. L. (1998). What immunity can protect against HIV infection? *J. Clin. Invest.* **102**, 1643-1644.
- LINCOLN, C. & NORDSTROM, R. (1976). Sabin polio vaccine for herpes simplex. *Schoch Lett.* **26**, 17.
- LINIAL, M. L. (1999). Foamy viruses are unconventional retroviruses. *J. Virol.* **73**, 1747-1755.
- MAULER, R., MERKLE, W. & HILFENHAUS, J. (1987). Inactivation of HTLV-III/LAV, hepatitis B and non-A/non-B viruses by pasteurization in human plasma protein preparations. *Dev. Biol. Stand.* **67**, 337-351.
- MELLERT, W., KLEINSCHMIDT, A., SCHMIDT, J., FESTL, H., ELMER, S., ROTH, W. K. & ERFLE, V. (1990). Infection of human fibroblasts and osteoblast-like cells with HIV-1. *AIDS* **4**, 527-535.
- MELTZER, M. S. & GENDELMAN, H. E. (1992). Mononuclear phagocytes as targets, tissue reservoirs, and immunoregulatory cells in human immunodeficiency virus disease. *Curr. Topics. Microbiol. Immunol.* **181**, 239-263.
- MOYER, M. P., HUOT, R. I., RAMIREZ, JR., A., JOE, S., MELTZER, M. S. & GENDELMAN, H. E. (1990). Infection of human gastrointestinal cells by HIV-1. *AIDS Res. Hum. Retroviruses* **6**, 1409-1415.
- MYERS, G. (1994). Tenth anniversary perspectives on AIDS. HIV: between past and future. *AIDS Res. Hum. Retroviruses* **10**, 1317-1324.
- NAHMIAS, A. J., WEISS, J., YAO, X., LEE, F., KODSI, R., SCHANFIELD, M., MATTHEWS, T., BOLOGNESI, D., DURACK, D., MOTULSKY, A., KANKI, P. & ESSEX, M. (1986). Evidence for human infection with an HTLV III/LAV-like virus in Central Africa, 1959. *Lancet* **1**, 1279-1280.

- NEUMANN-HAEFELIN, D., FLEPS, U., RENNE, R. & SCHWEIZER, M. (1993). Foamy viruses. *Intervirology* **35**, 196–207.
- PIOT, P., QUINN, T. C., TAELEMAN, H., FEINSOD, F. M., MINLANGU, K. B., WOBIN, O., MBENDI, N., MAZEBO, P., NDANGI, K., STEVENS, W., MITCHELL, S. & MCCORMICK, J. B. (1984). Acquired immunodeficiency syndrome in a heterosexual population in Zaire. *Lancet* **2**, 65–69.
- PLOTKIN, S. A., LEBRUN, A., COURTOIS, G. & KOPROWSKI, H. (1961). Vaccination with the CHAT strain of type 1 attenuated poliomyelitis virus in Leopoldville, Congo: 3. Safety and efficacy during the first 21 months of study. *Bull. World Health Org.* **24**, 785–792.
- RICH, E. A., CHEN, I. S. Y., ZACK, J. A. LEONARD, M. L. & O'BRIEN, W. A. (1992). Increased susceptibility of differentiated mononuclear phagocytes to productive infection with human immunodeficiency virus-1 (HIV-1). *J. Clin. Invest.* **89**, 176–183.
- ROTTMAN, J. B., GANLEY, K. P., WILLIAMS, K., WU, L., MACKAY, C. R. & RINGLER, D. J. (1997). Cellular localization of the chemokine receptor CCR5. Correlation to cellular targets of HIV-1 infection. *Am. J. Pathol.* **151**, 1341–1351.
- SHAH, K. & NATHANSON, N. (1976). Human exposure to SV40: review and comment. *Am. J. Epidemiol.* **103**, 1–12.
- SMITH, P. D., MENG, G., SHAW, G. M. & LI, L. (1997). Infection of gastrointestinal tract macrophages by HIV-1. *J. Leukoc. Biol.* **62**, 72–77.
- STAHL-HENNIG, C., STEINMAN, R. M., TENNER-RACZ, K., POPE, M., STOLTE, M., MATZ-RENSING, K., GROBSCHUPFF, G., RASCHDORFF, B., HUNSMANN, G. & RACZ, P. (1999). Rapid infection of oral mucosal-associated lymphoid tissue with simian immunodeficiency virus. *Science* **285**, 1261–1265.
- STEVENS, C. E., ALTER, H. J., TAYLOR, P. E., ZANG, E. A., HARLEY, E. J. & SZMUNESS, W. (1984). Hepatitis B vaccine in patients receiving hemodialysis. Immunogenicity and efficacy. *N. Engl. J. Med.* **311**, 496–501.
- STEVENS, C. E., TAYLOR, P. E., RUBINSTEIN, P., TING, R. C., BODNER, A. J., SARGADHARAN, M. G. & GALLO, R. C. (1985). Safety of the hepatitis B vaccine. *N. Engl. J. Med.* **312**, 375–376.
- STINE, G. J. (1998). *Acquired Immune Deficiency Syndrome: Biological, Medical, Social, and Legal Issues*. 3rd Edn, pp. 323–326. Englewood Cliffs: Prentice-Hall.
- STRICKER, R. B. & GOLDBERG, B. (1997). Polio vaccines and retroviral contamination. *J. Infect. Dis.* **176**, 545.
- SZMUNESS, W. (1979). Large-scale efficacy trials of hepatitis B vaccines in the USA: baseline data and protocols. *J. Med. Virol.* **4**, 327–340.
- SZMUNESS, W., STEVENS, C. E., HARLEY, E. J., ZANG, E. A., OLESZKO, W. R., WILLIAM, D. C., SADOVSKY R., MORRISON, J. M. & KELLNER, A. (1980). Hepatitis B vaccine: demonstration of efficacy in a controlled clinical trial in a high-risk population in the United States. *N. Engl. J. Med.* **303**, 833–841.
- SZMUNESS, W., STEVENS, C. E., HARLEY, E. J., ZANG, E. A., TAYLOR, P. E. & ALTER, H. J. (1981a). The immune response of healthy adults to a reduced dose of hepatitis B vaccine. *J. Med. Virol.* **8**, 123–129.
- SZMUNESS, W., STEVENS, C. E., ZANG, E. A., HARLEY, E. J. & KELLNER, A. (1981b). A controlled clinical trial of the efficacy of the hepatitis B vaccine (Heptavax B): a final report. *Hepatology* **1**, 377–385.
- SZMUNESS, W., STEVENS, C. E., HARLEY, E. J., ZANG, E. A., ALTER, H. J., TAYLOR, P. E., DE VERA, A., CHEN, G. T. & KELLNER, A. (1982). Hepatitis B vaccine in medical staff of hemodialysis units: efficacy and subtype cross-protection. *N. Engl. J. Med.* **307**, 1481–1468.
- TAGER, A. (1974). Preliminary report on the treatment of recurrent herpes simplex with poliomyelitis vaccine (Sabin's). *Dermatologica* **149**, 253–255.
- TEMIN, H. M. & MIZUTANI, S. (1970). RNA-dependent DNA polymerase in virions of Rous sarcoma virus. *Nature* **226**, 1211–1231.
- VAN DE PERRE, P., ROUVROY, D., LEPAGE, P., BOGAERTS, J., KESTELYN, P., KAYIHIGI, J., HEKKER, A. C., BUTZLER, J. P. & CLUMECK, N. (1984). Acquired immunodeficiency syndrome in Rwanda. *Lancet* **2**, 62–65.
- VANGROENWEGHE, D. (1997). *AIDS in Africa*, Chapter 4. Berchem: EPO VZW.
- VOGEL, G. (1999). FDA weighs using tumor cell lines for vaccine development. *Science* **285**, 1826–1827.
- WERNER, A., WINSKOWSKY, G., CICHUTEK, K., NORLEY, S. G. & KURTH, R. (1990). Productive infection of both CD4+ and CD4- human cell lines with HIV-1, HIV-2 and SIVagm. *AIDS* **4**, 537–544.
- ZHU, T., KORBER, B. T., NAHMIA, A. J., HOOPER, E., SHARP, P. M. & HO, D. D. (1998). An African HIV-1 sequence from 1959 and implications for the origin of the epidemic. *Nature* **391**, 594–597.