neurons become cholinergic when placed in tissue culture^{1,7}. But this adrenergic-to-cholinergic switch is triggered by conditioned medium most easily in neurons from neonates and occurs earlier rather than later during the culturing process⁷⁻⁹. The adult coeliac neurons

we^{5,10} and others¹¹ have studied are not provided with conditioned medium and maintain their catecholaminergic phenotype for more than 2 months in culture. Nicotinic synaptic transmission therefore is not to be expected and has not been observed; only ATP fast synaptic potentials have been recorded during this time. McLachlan and Keast appear to disdain these purinergic synapses as an artefact of culture; we view such synaptic purity as a virtue rather than a vice for studying mechanisms of action of this 'new' fast neurotransmitter.

Annmarie Surprenant

Richard J. Evans

*Vollum Institute,
Oregon Health Sciences University,
Portland,
Oregon 97201, USA*

1. McLachlan, E. M. & Keast, J. R. *Nature* **361**, 310 (1993).
2. Benham, C. D. *Nature* **359**, 103-104 (1992).
3. Burnstock, G. *Neurochem. Intl* **2**, 357-368 (1990).
4. Evans, R. J. & Surprenant, A. *Br. J. Pharmac.* **106**, 242-249 (1992).
5. Evans, R. J., Derkach, V. & Surprenant, A. *Nature* **357**, 503-505 (1992).
6. Edwards, F. A., Gibb, A. J. & Colquhoun, D. *Nature* **359**, 144-147 (1992).
7. Furshpan, E. J. et al. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4225-4229 (1976).
8. Johnson, M. I. et al. *J. Cell Biol.* **84**, 680-691 (1980).
9. Potter, D. D. et al. *J. Neurosci.* **6**, 1080-1098 (1986).
10. Vanner, S. et al. *J. Neurophysiol.* (in the press).
11. Coggan, J. S., Gruener, R. & Kreulen, D. L. *J. auton. nerv. Sys.* **34**, 147-156 (1991).

HIV vaccination dilemma

SIR — Ada *et al.* in their Commentary¹ discussed my paper² on the improbability of effective vaccination against human immunodeficiency virus (HIV) because of its intracellular transmission and rectal (or vaginal cervical) port of entry.

Neither antibodies nor cell-mediated immunity (CMI) is effective against intracellular transmission of HIV infection — CMI is directed against the viral antigens expressed on some HIV-infected cells but not on others in which HIV is suppressed as complementary DNA of HIV integrated in chromosomes. Ada *et al.* pointed out that such cells are, as a rule, foreign (allogeneic) to the recipient. Accordingly, they assumed that such allogeneic HIV-infected lymphocytes in the semen (or blood) would be rapidly destroyed by the recipient host cells, and that "if the HIV cDNA in live or dead semen cells were to be transferred undamaged in some novel way to the live host cells, as Sabin suggests, expression of viral peptides could also occur. If so, the 'transfected' host cells would be recognized by cytotoxic T lymphocytes (CTLs) in the vaccinated host." They added that "Cells in the donated semen already expressing viral antigen could fuse with host cells (gp120/CD4 interaction), and thus be recognized by both class I MHC restricted CTLs resulting from the vaccination and alloreactive CTLs."

The 'flaw' in this argument is that there is a "novel way" by which HIV-infected lymphocytes have been demonstrated to be capable of rapidly transferring infectious virus, either HIV RNA or chromosomally integrated HIV cDNA (without expressed viral antigens or peptides combined with class I MHC), in HIV-infected cells by cell-to-cell contact without using CD4 receptors. This phe-

nomenon was recently described by Phillips and Bourinbaier³, who showed that "contrary to infection with free virus, the cell to cell infection was not blocked by anti-gp120 or antiviral serum from HIV-positive individuals". These authors concluded that these "observations offer an insight into the cellular sequence of events which may take place during sexual transmission of HIV across an intact epithelial barrier." Even before HIV was identified as the cause of AIDS, Shearer⁴ suggested "allogeneic leukocytes as possible factors in induction of AIDS in homosexual men". Ada *et al.*, in downplaying "the efficacy of transmission of HIV infection by sexual intercourse, including receptive anal intercourse, and needle sharing" disregard the fact that in the United States in 1991, 64% of the reported cases of AIDS occurred in homosexuals, 6% in heterosexuals, and 23% among people involved only in intravenous drug abuse. The reported rapid transfer of HIV in its various intracellular states by cell-to-cell contact³, before the allogeneic mechanism can come into play, is the "novel way".

Also of relevance is the report⁵ that purified proviral DNA of simian immunodeficiency virus (SIV) is infectious *in vivo* but not *in vitro*. Ada *et al.*, in pointing to the effectiveness of some vaccines against intravenous challenge by cell-free virus, also overlook a report⁶ showing that vaccinated monkeys protected against an intravenous challenge with 25 monkey-infectious doses of cell-free SIV were not protected against a similar dose of intracellular SIV.

It is evident from the natural history of HIV infection in human beings that the antibody and cell-mediated immune responses are inadequate in preventing the accumulation in the lymph nodes and

other tissues of a reservoir of HIV-infected cells (many with only chromosomally integrated suppressed cDNA), which remain dormant for many years until they are activated (derepressed) by as yet incompletely understood events. Moreover, there is increasing recognition of mechanisms by which host immune defences are counteracted to produce latent and persistent infections by viruses⁷.

The continuing failure to use infected cells by various routes, rather than only cell-free virus by the intravenous route, to test for protection produced by vaccination was again evident in a report⁸ on the protective effect of a live attenuated SIV vaccine, with a deletion of the *nef* gene, in which two of four rhesus monkeys which previously resisted an intravenous challenge with ten cell-free monkey-infectious doses of virus, resisted a subsequent intravenous challenge with 1,000 cell-free monkey-infectious doses. The enthusiastic response of HIV vaccinologists to this finding⁹ shows the widespread naivety about what live virus vaccines cannot be expected to achieve. I said² that: "A live genetically engineered safe deletion mutant of HIV or SIV cannot be expected to do more than [natural infection with] the unmodified virus", which may perhaps be called Sabin's law about live virus vaccines.

There is no scientific evidence that any of the experimental vaccines against SIV or HIV, some of which are scheduled for use in large-scale human trials, have any protective effect against natural modes of transmission in previously uninfected or latently infected hosts. Ada *et al.* said that "to delay or not perform such trials for the reason proposed by Dr Sabin would be disastrous for the increasing numbers of people exposed to the risk of infection". In my judgement, it would be disastrous to continue the current inadequate methods of study of HIV and SIV vaccines, and to carry out large-scale tests in humans of vaccines without adequate evidence that such vaccines can protect against natural infection with adequate doses of intracellular virus.

Albert B. Sabin*

Washington DC 20016, USA

1. Ada, G., Blanden, B. & Mullbacher, A. *Nature* **359**, 572 (1992).
2. Sabin, A. B. *Proc. natn. Acad. Sci. U.S.A.* **89**, 8852-8855 (1992).
3. Phillips, D. M. & Bourinbaier, A. S. *Virology* **186**, 261-273 (1992).
4. Shearer, G. M. N. *Engl. J. Med.* **308**, 223-224 (1983).
5. Letvin, N. L. et al. *Nature* **349**, 573 (1991).
6. Johnson, P. R. et al. in *Vaccines 92: Modern Approaches to New Vaccines Including Prevention of AIDS* (eds Brown, F. et al. 95-102 (Cold Spring Harbor, 1992).
7. Gooding, L. R. *Cell* **71**, 5-7 (1992).
8. Daniel, M. D. et al. *Science* **258**, 1938-1941 (1992).
9. Cohen, J. *Science* **258**, 1880-1881 (1992).

* Dr Sabin died on 3 March. *Nature* will be printing an obituary shortly.