PROTECTION INDUCED BY SIV VACCINES IN RHESUS MACAQUES

Group	Type of vaccine	Type of i.v. SIV challenge (10 MID_{50})	Monkeys protected from viraemia*/ monkeys per group	Serum antibody titres measured in ELISA (log_{10} ±s.d.) at day of challenge† Antigen		
				SIV-env	C8166	RhPBMC
A	SIV–MDP	cell-free	4/4	2.4±0.4	2.0±0.2	1.9±0.1
	SIV–iscom‡	cell-free	3/3	3.4±0.2	2.7±0.2	2.7±0.2
	MV–MDP	cell-free	0/2	≤0.8	2.1±0.1	1.8±0.2
	MV–iscom	cell-free	0/2	≤0.8	1.9±0.1	1.9±0.3
B	SIV–MDP	infected PBMC	2/4	3.3±0.4	2.1±0.1	1.9±0.1
	SIV–iscom	infected PBMC	2/4	3.6±0.3	2.8±0.2	2.7±0.2
	MV–MDP	infected PBMC	0/2	≤0.8	≤1.5	1.9±0.3
	MV–iscom	infected PBMC	0/2	≤0.8	≤1.5	2.1±0.1

* Viraemia was demonstrated by co-cultivaiton of PBMC with C8166 cells and subsequent demonstration of SIV antigen in the medium by P27–antigen capture assay. Results were confirmed by showing serum antibody induction (MV-vaccinated monkeys) or booster reaction, in an SIV env-specific ELISA and Western blot assay.
† SIV–Env: inhibition of reactivity of labelled SIV-neutralizing mouse monoclonal antibody (KK5) by serial dilutions of monkey sera. KK5 kindly provided by Dr K. Kent (through MRC-ADP).
‡ One animal not included (died during recovery at day of challenge).
C8166/RhPBMC, reactivity of serial dilutions of monkey sera with solubilized membrane protein fraction (about 10 μg ml^{-1}) of respective cells attached to ConA-coated wells.

lar vaccination all the monkeys were challenged intravenously with either 10 monkey infectious doses (MID_{50}) $SIV_{MAC}251$ (32H) propagated in C8166 cells (group A), or with SIV-infected peripheral blood mononuclear cells (PBMC) obtained from an SIV-infected rhesus macaque (1×C) (group B). These PBMC had been prepared from heparinized blood of the monkey, 11 months after experimental infection with the same cell-free challenge stock of SIV_{MAC} 251 (32H), frozen in 5×10^5 cell aliquots and subsequently titrated *in vivo* in rhesus macaques. The equivalent of 10 MID_{50} of these cells was used as challenge dose for group B.

The results are shown in the table. All the monkeys of group A vaccinated with SIV–MDP or SIV-iscom were protected from developing SIV viraemia for the 12-week observation period after intravenous cell-free $SIV_{MAC}251$ (32H) challenge, whereas all the MV–MDP and MV-iscom vaccinated monkeys developed SIV viraemia within 4 weeks after receiving the same cell-free challenge. Also all the MV–MDP and MV-iscom vaccinated monkeys of group B developed SIV viraemia within 2 weeks after intravenous challenge with SIV-infected PBMC. Two out of four SIV–MDP vaccinated monkeys and two out of four SIV-iscom vaccinated monkeys of group B were protected from SIV viraemia for the 9-week observation period after intravenous challenge with the SIV-infected PBMC.

These data were confirmed by demonstrating that all the monkeys that became SIV-viraemic also showed booster responses in their serum antibody titres measured in an SIV env-specific enzyme-linked immunosorbent assay (data not shown). At the day of challenge, all SIV-vaccinated animals had developed serum antibody titres in this assay. In addition, antibodies directed against C8166 cells and rhesus PBMC present at that day were measured (see table). The protection found in group B among SIV-vaccinated monkeys did not correlate with the levels of serum antibody titres to C8166 cells or PBMC in these animals.

This is the first demonstration in the SIV-macaque model that vaccination can protect against challenge with cell-associated SIV, and is the first report that vaccinated, previously unchallenged non-human primates can be protected from infected PBMC from another infected animal. As the SIV challenge material used in group B was directly prepared from infected PBMC of the homologous species, the partial protection observed against this severe intravenous challenge should be attributed to immunization with SIV-specific antigens. Consequently the suggestion by Stott *et al.*[1], that virus- as well as cell-specific components can still be involved in protection after vaccination with inactivated SIV preparations, is strongly supported by our observations.

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SIR — Stott *et al.*[1] have presented evidence that the protection elicited with an SIV vaccine[9] containing fixed SIV-infected cells of human origin (C8166) may, at least in part, be mediated by anti-cell responses resulting from xeno-immunization. This surprising observation raises important questions about the nature of the protective responses elicited by other SIV vaccines.

As part of an AIDS vaccine development project, within the MRC AIDS Directed Programme and the European Communities' Concerted Action programme, we have immunized 10 rhesus macaques with a partially purified, inactivated virus vaccine based on the 32H isolate of SIV_{MAC} strain 251 grown in C8166 cells (manuscript in preparation). Four animals (group A) each received four 500-μg doses of vaccine given in the Syntex adjuvant formulation 1 (saf-1). Another two groups of three animals each received the same vaccine given in alum, either as four 500-μg doses (group B) or as four 100-μg doses (group C). All animals were challenged intravenously 2 weeks after the final vaccine boost with 10 median monkey infectious doses (MID_{50}) of cell-free virus. Group A were challenged with $SIV_{SM}B670$, a related but antigenically distinct strain of SIV, grown on human peripheral blood mononuclear cells (PBMC)[8]. Animals in groups B and C were challenged with homologous virus, that is the 32H isolate of $SIV_{MAC}251$ grown on C8166 cells.

Although unvaccinated control animals all became infected, all of the vaccinated animals were protected from infection as determined by the inability to recover virus from PBMC, inability to detect proviral DNA in PBMC and lack of an anamnestic antibody response. Animals were subsequently boosted further with vaccine, formulated as previously, at 16 months (group A) and 6.5 months (groups B and C) after initial challenge. At 2 weeks after the additional boost, all 10 animals plus 4 unvaccinated controls were challenged with 10 MID_{50} of cell-free $SIV_{MAC}251$ grown in monkey PBMC. Virus was recovered from all of these animals as early as 2 weeks after challenge. Thus no protection from challenge was elicited despite the fact that the additional vaccination boosted the SIV-specific antibody response. In this experiment we have not

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formally established if the animals would have been protected from rechallenge with SIV grown in human cells, but in previous experiments we have obtained protection following reboosting.

At first sight our results suggest that the challenge viruses used in these experiments may have acquired properties, either by genetic selection or, perhaps more likely, epigenetically, from the cells in which they were grown. Thus anti-cellular responses elicited by immunization may have been responsible for the protection observed when both vaccine and challenge virus were derived from the xenogeneic human cells. But caution is necessary in interpreting these results: because the virus used for rechallenge was produced in rhesus monkey PBMC from the original $SIV_{MAC}251$ stock, it could have contained variants that were critically divergent from the cognate virus used to prepare the vaccine.

It is indeed remarkable that crude inactivated SIV vaccines provide complete protection against challenge with various antigenically distinct viruses which, in unvaccinated animals, invariably induce AIDS. However, this failure to protect against virus grown in monkey PBMC and the significance of anti-cellular responses must be understood so that the protecting machanisms can be defined and applied to AIDS vaccine development.

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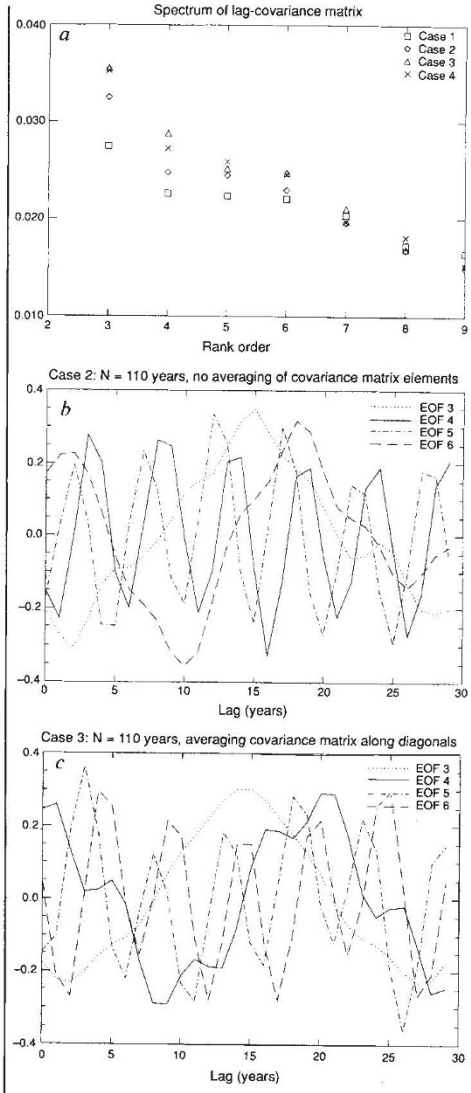
Temperature time-series?

SIR — Ghil and Vautard[1] applied the method of singular-spectrum analysis to a 135-year time-series of global annual-average near-surface temperatures, and found a pair of high-variance empirical orthogonal functions (EOFs) characterizing a temperature oscillation with a period of slightly more than 20 years. Elsner and Tsonis[2] repeated the analysis considering the most recent 130-, 110- and 90-year segments of the time-series. They report that the EOF 'signature' of the 20-year oscillation appears only if all 130 years are included, suggesting it may be an artefact of the (unreliable) early part of the time-series.



a, Portion of spectrum of covariance matrix constructed[1,2] from $M = 30$ lagged copies of the IPCC consensus time-series of global temperatures[3]. Note the linear scale, to emphasize the similarity of eigenvalues 4, 5 and 6 in all cases considered. Ordinates: *a*, eigenvalue; *b*, *c*, eigenvector. *b*, EOFs 3–6 for case 2: $N = 110$ years, no averaging of covariance matrix elements. Note EOFs 3 (dotted line) and 6 (dashed line) are in quadrature, indicating a 20–30-year oscillation, while EOFs 4 (solid line) and 5 (dash-dot line) indicate a 5–6 year oscillation. *c*, EOFs 3–6 for case 2: $N = 110$ years, averaging covariance matrix elements along diagonals. Note EOFs 3 and 4 are now in quadrature.

We found this pair of results intriguing, because Ghil and Vautard also show a reconstruction of the original data by projection onto a number of subsets of their EOF eigenbasis, including one in which only the lowest 4 EOFs (which include the 20-year oscillatory pair) are retained (their Fig. 3*a*). The amplitude of the 20-year oscillation does not appear to diminish in the later part of the time-series, as would be expected if the conclusion of ref. 2 (that all the power in this pair of EOFs is confined to the earlier part) were correct. Using the IPCC consensus time-series[3] (data kindly provided by D. E. Parker), we reproduced both sets of results. Are they a paradox?

The answer is no. If we consider the relevant portion of the spectrum of the lag-covariance matrix, including all 130 years (*a* in the figure, case 1), we notice that the eigenvalues of EOFs 4, 5 and 6 are remarkably similar. A small change in analysis technique or time-series length might therefore change the order in which these EOFs appear. Sure enough, if we consider only the last 110 years (*a* in the figure, case 2), and inspect EOFs 3 to 6 (*b*), we notice that EOF 6 (dashed line) is in quadrature with EOF 3 (dotted line), forming a 20–30-year oscillatory pair, whereas with the full 130-year time-series, it is EOFs 3 and 4 which are in quadrature (Fig. 1*b* of ref. 1). Thus, removing the first 20 years of the time-series causes EOFs 4 and 6 to exchange positions in the eigenvalue rank-ordering, through a very slight change in their respective eigenvalues.

Ghil and Vautard impose a Toeplitz structure on the lag-covariance matrix. We are unsure if this is advisable, as it imposes symmetries on the problem which may not be present in a short data series. But if we do the same (*a*, case 3), we can make EOF 4 and EOF 6 swop back again (*c*). If we consider only the last 90 years of the series, they re-exchange. If the rank order of these EOFs is so sensitive to details of the analysis, the order itself cannot have any physical significance. In dealing with such a short time-series, the possibility that results may be a consequence of a few anomalous decades, or an artefact of time-series and window lengths, must always be considered carefully. But Elsner and Tsonis have definitely failed to prove their case against the 20-year oscillation.

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