Epstein–Barr virus

Dream or reality of a vaccine?

from A.J. Beale

EPSTEIN–BARR virus (EBV) was first recognized in cultures of Burkitt's lymphoma cells and is a member of the herpes virus family. It was soon established as a cause of infective mononucleosis. There has also been a strong association between the virus, Burkitt's lymphoma and nasopharyngeal carcinoma. Since 1976, Epstein and his colleagues have mounted a systematic search for a vaccine against the virus in the hope that the viral aetiology of the two malignancies could be proved, with much benefit to high-risk populations in Africa, China and South-East Asia. Their studies have reached another landmark, as described on page 287 of this issue of *Nature*.

Epstein *et al.* have previously identified as a candidate immunogen an EBV glycoprotein termed gp340, and have devised means for its extraction, techniques for measurement of both gp340 and antibodies to it, and have developed an animal model of EBV-induced lymphomas in cottontop tamarins (*Saguinus oedipus oedipus*). The stage was set for the critical experiment in which vaccinated and unvaccinated tamarins were challenged with EBV. This experiment and the success of the immunization is now reported.

As the test vaccine, gp340 preparations, containing a minimal amount of residual living virus, were given to two tamarins in eight intraperitoneal doses at fortnightly intervals. Both animals developed antibodies that could be detected by an enzyme-linked immunosorbant assay, by immunofluorescence and by neutralization of EBV transformation of cord blood cells. They were both protected against a massive challenge dose of virus which caused disease and multiple tumours in control animals. Isolated gp340 in liposomes was less immunogenic than the membrane preparation: in a group of four animals given this vaccine, only one produced high concentrations of antibodies and was protected against viral challenge. Thereafter, two animals were given 17 intraperitoneal doses of the liposome preparation at fortnightly intervals and were subsequently challenged. Both animals developed transient inguinal lymph node enlargement after challenge; in one case this was accompanied by transient mesenteric node enlargement.

From the successful development of a vaccine against Marek's disease, to which fowl are particularly prone, it is already clear that a vaccine against tumours caused by herpes viruses is feasible, and the work now being reported on EBV shows that the development of a similar vaccine for a human herpes virus is theoretically possible. Is it likely to be a practical proposition to make and test such a vaccine? The candidate vaccines of Epstein *et al.* are not practical: clearly, the membrane preparation of gp340 would need rigorous testing to show it was free of infectious virus before its use in man could be contemplated, and either vaccine would need to be presented in such a way that a protective immune response is achieved with fewer doses of vaccine. Doubtless the schedule could be improved to ensure priming and the elicitation of a secondary response.

The most important advance referred to by Epstein and his colleagues is the cloning and sequencing of EBV and identification of the sequences encoding gp340. It should therefore be possible to produce the protein in large quantities. Since it is glycosylated — more than 50 per cent of the mass is carbohydrate — it may prove best to prepare it in mammalian cells, as is done for many other viral immunogens, rather than in bacteria or yeast. Alternatively it could be expressed in vaccinia virus or some other carrier. The groundwork for developing a vaccine against EBV seems to have been soundly laid and the technology to produce sufficient immunogen is at hand.

The major problems of organizing and financing the procurement and testing of such a vaccine remain to be solved. The problems of organizing trials against diseases caused by EBV are formidable, even when compared with other herpes virus infections. Whereas infectious mononucleosis is an early manifestation of primary infection, Burkitt's lymphoma and nasopharyngeal carcinoma are late manifestations that often take many decades to emerge. No one knows at present whether the late manifestations can be prevented after infection, but current opinion is sceptical. The aim, therefore, must be to prevent infection.

Trials of a vaccine against infectious mononucleosis are a practical and worthwhile proposition, given the will, in Western countries. Proof that EBV infection and this disease can be prevented by a vaccine would warrant the use of a vaccine to prevent Burkitt's lymphoma and nasopharyngeal carcinoma. It is doubtful whether it is practical to carry out a placebo-controlled trial lasting decades, but observation of the effect of vaccine on disease together with more limited trials of the vaccine on viral infections may suffice to demonstrate that EBV is indeed the cause of Burkitt's lymphoma and nasopharyngeal carcinoma.

For a number of diseases, ranging from malaria and pertussis to those caused by hepatitis B and EB viruses, there are now prospects for control by immunization, based on a molecular understanding of the immunogen required to produce protection. To harness this promise, a more determined and imaginative approach to preventive medicine and public health is required. Provided government agencies can see the economic as well as the health benefits of developing such approaches, the benefits to mankind and human health could be immense. □

A.J. Beale is at Wellcome Biotechnology Ltd., Beckenham, Kent BR3 3BS, UK.

γ-ray sources

Does Geminga exist yet?

from Roger W. Romani and Virginia Trimble

THE peculiar γ-ray source in the constellation Gemini, termed Geminga, is once again puzzling astronomers. Originally found by the SAS-2 satellite[1] and later studied by γ-ray detectors both in space[2] and on Earth, this object presents strange features in every wavelength band. One of the strangest — that it cannot be detected at visible and radio wavelengths — is reflected in its name, which also means "does not exist" or "is not there" in Milanese dialect. New optical observations (refs 4–6 and G.F. Bignami *et al.*, in preparation) have provided a tentative identification but these make Geminga seem even more inexplicable: as G.F. Bignami (Milan) reported at a recent meeting*, the object is exceedingly faint and may have a very large proper motion.

As the brightest of the 20 unidentified γ-ray sources in the COS-B catalogue[2], Geminga is a natural subject of searches for corresponding sources in other energy ranges. The high count rate and large distance from the confusion at the galactic centre allowed the COS-B collaboration to obtain a position that is excellent by γ-ray standards. Images taken with the Einstein X-ray satellite's high resolution imager (HRI) and imaging proportional counter (IPC) led to the identification of Geminga with the bright source 1E0630+178 (ref.7), supported, it then seemed, by the same 59–60-second, gradually lengthening pulse period in both X- and γ-ray data[8]. Buccheri *et al.*[9] and others have doubted the periodicity, without necessarily disbelieving the identification, because there is unlikely to be an unusual

*NATO Advanced Study Institute on "High Energy Phenomena around Compact Stars" held at Cargese, Corsica, 2–13 September, 1985.

X-ray source and a bright γ-ray source in one 0.4 square degrees of sky.

Searching at lower frequencies and with greater positional accuracies has met with some difficulty. Radio maps of the γ-ray error box (refs 10–12 and V. Boriakoff *et al.*, unpublished data) reveal more than a dozen 10–100 mJy sources, but there is nothing at the X-ray position and nothing particularly unusual anywhere.

At optical wavelengths, deep charge-coupled device exposures taken at the Canada–France–Hawaii telescope in January 1984 revealed a 21st magnitude image marginally within the Einstein error circle[13]. Dubbed the G candidate, it has been closely scrutinized by a number of groups. Discouragingly, optical and infrared photometry and spectroscopy (refs 4,5,14,15 and M.J. Lebofsky, unpublished data) indicate that the candidate probably is just a G-type star — a cool white dwarf 100–200 pc from us or, more likely, a slightly hotter main-sequence or subdwarf star several thousand parsecs away. Both are inconsistent with the Einstein X-ray colour temperature of about 10^6 K (ref. 7) and the latter is also inconsistent with the very low X-ray absorption ($\leq 2 \times 10^{20}$ H cm^{-2}), which indicates a distance less than 250 pc.

Moreover, a careful search for optical pulsations at a range of periods around those reported in X and γ-rays[5], has set 2–4 per cent limits on any variability of the G candidate. The sky, according to Lebovsky, is full of similar stars and the chances of this one having anything to do with Geminga are slim.

Meanwhile, back at Lick Observatory, Djorgovski and Kulkarni[4] have searched deeper at the HRI position and found two fainter candidates: G′ at 24·5 mag near the centre of the error circle and G″ at 25½–26 mag off to one side. The lack of any brighter optical counterpart has interesting implications for 1E0630+178. The ratio of X-ray to optical luminosity is about 1,000 if G′ is the counterpart, 2,500 if G″, and ≤ 3,000 if neither is — among identified Einstein sources, only the radio pulsars and low-mass X-ray binaries have such large ratios. The absence of a detectable radio signal argues against a radio pulsar and the faintness of G′ would place a typical X-ray binary at the improbable distance of 200,000 pc, arguing against an X-ray binary. This makes 1E0630+178 unlike any other known X-ray source, whatever association it has with Geminga.

G′ and/or G″ seem to be peculiar, in any case. Djorgovski and Kulkarni[4] report that G′ has a tentative proper motion of 0.6 seconds per year; an alternative explanation is that this reflects a larger motion of G″ plus spillover of light between the images. In addition, the image that Bignami and his collaborators have obtained by summing the twelve exposures made in January 1984 shows a faint object that could be G″. But its position is very different from that found at Lick in March 1985, implying a proper motion of 1.4 seconds per year for G″ and an upper limit of ≤ 0.2 seconds per year for G′.

Its rapid motion, if confirmed, must place the star quite close to us; for example, even a halo object moving at 200 km s^{-1} would be no more than 30 pc away. What could it be? From the magnitudes, G′ and G″ could be interpreted as brown dwarfs, not unlike the recently-discovered companion of vB8 (ref. 16). But such a star is most unlikely to be either the X-ray source or a chance superposition; if there were a single brown dwarf so close to us in every 10^5 Einstein error circles (r = 3.3″), it would make up the entire local 'missing mass' and can therefore be excluded from searches of larger areas.

It is somewhat more likely that G′ or G″ is the thermal emission from a neutron star. Because the optical effective temperature could be anywhere from the X-ray colour temperature (10^6 K at the hot polar caps) to the X-ray effective temperature ($\sim 3 \times 10^5$ K), the implied distance is quite uncertain. Possible combinations are G″ with the lower temperature and a runaway velocity of about 200 km s^{-1} at 20–30 pc, or G′ with the higher temperature and atypical young star velocity of ≤ 50 km s^{-1} at 80–100 pc.

Not even a white-dwarf binary companion[17,18] could remain hidden at these distances, which leaves only binary[19] or single[20] neutron stars as viable models. Either is possible in energetic terms, if the 60-second period is real and can be attributed to orbital motion or rotation in the two cases, although this then means γ rays must be grossly non-thermal. But both models imply soberingly short lifetimes, about 700 years, and the second may also have difficulties in explaining the acceleration of particles to the energies needed to emit γ rays. The short lifetime would make sense if the 'guest star of +437 gave birth to Geminga[8] but the total absence of emission nebulosity in the vicinity[4] argues against any recent local violence.

Clearly, one or two more deep images of the region, confirming or refuting the rapid motion of G″ and providing a bit of colour (temperature) information on the two candidates, will be critical for sorting out the confusion, in which Geminga, 1E0630+178 and G′ or G″ could be one, two or three interesting objects. □

1. Fichtel, C.E. *et al. Astrophys. J.* **198**, 163 (1975).
2. Swanenburg, B.N. *et al. Astrophys. J.* **243**, L69 (1981).
3. Zyskin, Y.L. & Mukanov, D.B. *Sov. AJ Lett.* **9**, 117 (1983).
4. Djorgovski, S. & Kulkarni, S.R. *Astr. J.* (in the press).
5. Kulkarni, S.R. & Djorgovski, S. *Astr. J.* (in the press).
6. Vigroux, L., Paul, J., Delache, P., Bignami, G.F. & Careveo, P.A. *18th ESLAB Symp.* (1985).
7. Bignami, G.F., Caraveo, P.A. & Lamb, R.C. *Astrophys. J.* **272**, L9 (1983).
8. Bignami, G.F., Caraveo, P.A. & Paul, J.A. *Nature* **310**, 464 (1984).
9. Buccheri, R., D'Amico, N., Hermsen, E. & Sacco, B. preprint.
10. Sieber, W. & Schlickeiser R. *Astr. Astrophys.* **113**, 314 (1982).
11. Spelstra, T.A. & Hermsen, W. *Astr. Astrophys.* **135**, 135 (1984).
12. Caraveo P.A. *et al. Adv. Space Res.* **3**, 77 (1984).
13. Caraveo, P.A., Bignami, G.F., Vigroux, L. & Paul, J.A. *Astrophys. J.* **276**, L45 (1984).
14. Sol, H. *et al. Astr. Astrophys.* **144**, 109 (1985).
15. Halpern, J.P., Grindlay, J.E. & Tytler, D. *Astrophys. J.* **296**, 190 (1985).
16. McCarthy, D.W., Probst, R. & Low, F. *Astrophys. J.* **290**, L9 (1985).
17. Bisnovatyu-Kogan, G.S. *Nature* **315**, 555 (1985).
18. Arons, J. preprint.
19. Nulsen, P.E.J. & Fabian A.C. *Nature* **312**, 48 (1984).
20. Katz, J. *Astrophys. Lett.* **24**, 183 (1985).

Roger W. Romani is at the California Institute of Technology, Pasadena, California 91125; Virginia Trimble is at the University of Maryland, Maryland 20742 and University of California, Irvine, California 92717, USA.

Plant sciences

Molecular view of pollen rejection

from Deborah Charlesworth

In many species of flowering plants, a genetic self-incompatibility mechanism causes the rejection of pollen from the same plant. The genetic control is determined by one or more self-incompatibility (*S*) loci and it has been clear for several years that many of the mysteries surrounding the *S* locus will be resolved only when the molecular structure of the locus is known. A start in that direction is provided by J.B. Nasrallah and colleagues on page 263 of this issue[1].

Two types of self-incompatibility are known[2]. In the Compositae and Cruciferae, and perhaps also in some other families[3-5], control of the pollen reaction is sporophytic — that is, the pollen type is determined by the genotype of the plant that has produced it — and there is usually, perhaps invariably, a single *S* locus. In gametophytic systems, which are known in several families and where the pollen type is controlled by the alleles in the pollen grains, there is often one but may be two *S* loci, as in the grasses, or even more; *Beta vulgaris* has four[6]. With both types of system, there are very large numbers of alleles at the *S* loci, so that although the genetic data indicate that the *S* loci control the specificity of the reactions, which must be expressed both in pollen and in the stigma or style in order for self-incompatibility to result, there has been a recurring tendency to doubt whether the sequence information for all the S glycoproteins is really encoded at the *S* locus, and to suggest that some or all of it must exist elsewhere in the genome, and that the *S* locus somehow switches between specificities[2,7].

Nasrallah *et al.* already have some data relevant to this question. They have cloned DNA from the S_6 allele of *Brassica oleracea* and shown that the protein encoded by the *S* DNA is detected by an