insuperable.

Our understanding of speciation has progressed depressingly little since the classic reviews by Dobzhansky[1] and Mayr[9]. This is partly because of the difficulties of studying a historical process. But it also results from the preoccupation with schemes such as sympatric and chromosomal speciation, and speciation caused by genetic drift, that have little theoretical or empirical support and are considered infrequent even by their proponents. Like Wright's shifting balance theory, these processes may operate in nature and may even be demonstrable in the laboratory, but in nature are hard to distinguish from better-established alternatives.

Nevertheless, there are some questions that seem more tractable. How many genetic changes separate two species? Although there are straightforward methods for mapping genes causing reproductive or morphological differences, there have been comparatively few genetic analyses of closely related species. In most animals, reproductive isolation stems from changes at many genes, but speciation in plants may often involve very few loci[10]. Are there other differences between plant and animal speciation? The Hawaiian *Bidens* show that substantial adaptive divergence can occur without reproductive isolation, perhaps because of the compartmentalized development of plants. What is the normal function of genes that cause reproductive isolation? Mapping should soon permit cloning and sequencing, and provide clues to their normal role in development. More attention should be devoted to the possibility of speciation through sexual selection, where the connection between selection and reproductive isolation is clear. Are there 'rules' of speciation that apply across groups? The ubiquity of Haldane's rule in animals (the preferential sterility of heterogametic hybrids) and its likely origin by rapid evolution of the sex chromosomes[11] are two patterns without widely accepted evolutionary explanations. Comparative studies may reveal other such patterns and underscore the need for good systematics and accurate estimates of divergence times. A combination of genetic and developmental approaches may offer the most progress in understanding the evolution of reproductive isolation. Without such knowledge, we are simply unable to evaluate the many theories of speciation. □

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J. A. Coyne is at the Department of Biology, University of Chicago, Illinois 60637, USA, and N.H. Barton is at the Department of Genetics and Biometry, University College London, London NW1 2HE, UK.

Vaccine development

Which way for malaria?

F.E.G. Cox

THE discovery, recently reported[1] by Louis Schofield and collaborators, that immunity against malaria operates against stages of the parasite developing in the liver calls for a radical reappraisal of the ways in which malaria vaccines are being developed, and at the same time explains several paradoxes.

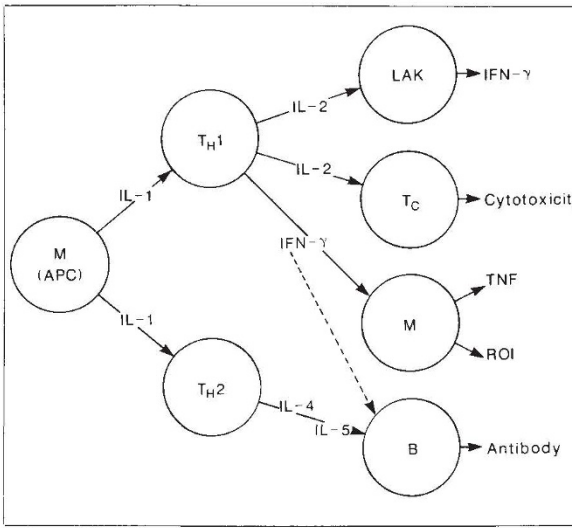
The life-cycle of the malaria parasite begins when a mosquito injects infective stages, called sporozoites, directly into a blood capillary. Sporozites enter liver hepatocytes within minutes and undergo a massive phase of asexual multiplication that results in the formation and release of up to 30,000 merozoites. These invade red blood cells and undergo further cycles of multiplication, destroying increasing numbers of erythrocytes. Sporozoites, liver-stages, merozoites and intraerythrocytic stages of the malaria parasite possess largely unique repertoires of antigens and, although clinical immunity is associated with the blood stages, the sporozoite is the obvious target for a vaccine.

Attempts to develop vaccines against sporozoites and blood stages have concentrated on the identification and synthesis of dominant surface proteins[2], but despite showing initial promise, this approach has been largely disappointing. Recombinant or synthetic sporozoite antigens protect some but not all of the volunteers[3,4], and the immunization of owl monkeys with a major blood-stage antigen of the malaria parasite *Plasmodium falciparum* was only partially effective[5].

There are some reservations about sporozoite and blood-stage vaccines. The main arguments against a sporozoite vaccine are that only one sporozoite needs to escape to the liver to initiate an infection, and that in endemic areas people acquire high levels of anti-sporozoite antibodies but still become infected. An argument against merozoite or blood-stage vaccines is that although they might ameliorate clinical disease, they cannot prevent infection. The liver stages of the parasite have received relatively little attention because it has been widely believed that no immunity to these intracellular forms exists. The recent work by Schofield *et al.*[1] shows this assumption to be false, and also points to the central involvement of antibody-independent immune mechanisms in immunity to malaria.

Development of the liver stages of *P. berghei*, a malaria parasite of rodents, can be prevented *in vivo*[6] and *in vitro*[7] by administration of minute quantities of gamma-interferon (IFN-γ) — but if this is a transient phenomenon it is unlikely to be of any practical importance. Schofield *et al.*, also using *P. berghei*, treated rats immunized with attenuated sporozoites, a very effective method of vaccination, with a monoclonal antibody capable of neutralizing IFN-γ. This treatment not only permits infection of liver cells but also reverses established sterile immunity. The protective effect of IFN-γ is therefore not transient, but is an integral part of the immune response to malaria.

Several points concerning vaccination emerge from these experiments. Protective vaccination can be achieved by



Part of the immunoregulatory circuit. Parasite antigens are processed by macrophages (M) which act as antigen-presenting cells (APC). The first signal is interleukin-1 which activates two kinds of helper T-cells (T_H1 and T_H2). Further signals such as interleukin-2 (IL-2) activate lymphokine-activated killer cells (LAK) or cytotoxic T-cells while gamma-interferon (IFN-γ) activates further macrophages, and interleukins 4 and 5 (IL-4 and IL-5) activate B-cells. The net result is the production of cytotoxic T-cells, antibody, IFN-γ, tumour necrosis factor (TNF) and reactive oxygen intermediates (ROI), which separately or together can destroy malaria parasites. Interferons can also inhibit B-cell activity (broken line).

immunization with sporozoites or sporozoite surface antigens, and this protection probably involves antibodies. But similar vaccines are also effective in B-cell-depleted mice[8], so antibody cannot be solely responsible for the induction of immunity. The depletion of $CD8^+$ (suppressor/cytotoxic) T-cells also reverses immunity[1], which implies either that $CD8^+$ cells might be cytotoxic, in which case they could kill malaria-infected hepatocytes by the same mechanism as they do in cattle infected with *Theileria parva*, another protozoan[9]; or that $CD8^+$ T cells might be the source of the IFN-γ now known to inhibit the development of the liver stages.

It is unlikely that IFN-γ alone is an effector molecule in immunity to malaria. Elucidation of the immune response as a whole is beginning to revolve around our understanding of the various intercellular signals involved. Although the figure shows only some pathways, it is clear that IFN-γ could be involved either as a product of LAK cells[10] or as a signal from TH1 cells[11] on macrophages triggering the production of reactive oxygen intermediates and/or tumour necrosis factor. A role for reactive oxygen intermediates in immunity to the erythrocyte stages of malaria has long been argued by Clark and his colleagues[12], and it would not really be surprising if immunity to the liver stages of the malaria parasite also involved a similar process, probably in combination with other mechanisms.

The possibility of developing a malaria vaccine has come a little closer as a result of the work of Schofield *et al.*, but it will not be straightforward. It is necessary to take into account the whole immune response, including its various regulatory hormones, and to manipulate these to drive the immune response along a desired pathway, perhaps by adding vectors containing interleukin-2[13] or helper T-cell epitope[14]. It will not be sufficient to hope for an additive effect of humoral and cellular components of the immune system, because these may not act in a synergistic way. □

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F.E.G. Cox is in the Department of Biophysics, Cell and Molecular Biology, King's College London (KQC), 26-29 Drury Lane, London WC2B 5RL, UK.

Quantum chemistry

Helium can form stable bonds

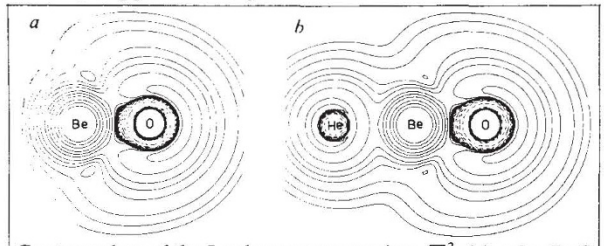
Neil Bartlett

Of all the noble gases, helium is the most noble. No neutral molecules are known that contain a helium atom, although helium does form ionic molecules and, in its excited states, transient 'excimer' molecules. In a recent paper, however, W. Koch *et al.* (*J. Am. chem. Soc.* **109**, 5912–5934; 1987) demonstrate persuasively with results from high-level computations that the linear molecule HeBeO is stable with respect to dissociation into helium and BeO, each species being in its ground state. The enthalpy for that process is endothermic by about 14 kJ mol^{-1}.

The first ionization potential of the helium atom (24.6 eV) is the highest known, approximately twice that of xenon. The known (weak) bonding capability of xenon depends on electron donation (of which the ionization potential is a useful measure) to the atom or atoms to which it is bonded, and helium must, by comparison, be a much less effective electron donor. Indeed, it is the least likely species to form dative bonds. The high-level quantum-mechanical evaluations of Koch *et al.* show that BeO is an effective acceptor at the beryllium atom because that atom has effectively given its valence electrons to the oxygen atom in bonding to it. Consequently the BeO presents a hole of cylindrical (σ) symmetry at its beryllium end, and to an approaching helium atom that end appears to be approximately Be^{2+}. All other electrons, which would have repulsive interactions with the helium electrons, are located on the oxygen atom and are consequently, in linear HeBeO, as far removed as possible from the He–Be bond.

In the Hartree–Fock molecular-orbital calculations, a rather large basis set (83 independent basis functions) was used to describe the wave function of the 14 electrons and fourth-order perturbation theory was used to account for the electron-correlation energy. Almost the same results were obtained at the multiconfiguration self-consistent-field level with a smaller basis set (35 basis functions). The essence of these long and detailed quantum calculations is to find the lowest energy distribution of electrons in the molecule. Also, the effect of zero-point energy was taken into account to evaluate enthalpies. Koch *et al.* show that helium forms strong bonds if the binding partner (acceptor) provides low-lying empty σ-orbitals. Electronegative atoms such as fluorine or oxygen are not suitable partners because they are electron rich. For similar reasons, HeOBe is not bound.

In its ground state (denoted $^1\Sigma^+$), BeO has a pair of core electrons on each atom ($1\sigma^2$, $2\sigma^2$), a triple bond ($3\sigma^2$, $1\pi^4$) and a non-bonding lone pair ($4\sigma^2$). The interaction with helium occurs through the lowest unoccupied molecular orbital, 5σ. Koch *et al.* find that HeBeO is stable at all levels of theory. The figure compares the charge distribution in BeO and HeBeO. The Laplace concentration (which represents the second derivative of the charge density with respect to the coordinates) is used to give greater contrast to the areas of charge concentration and charge depletion in the molecules, relative to the neutral atoms. The picture of BeO shows that the beryllium has donated valence-shell electrons to oxygen, but that of the HeBeO reveals barely any further deformation of the electronic structure of the BeO and helium participants.



Contour plots of the Laplace concentration, $\nabla^2\rho(\mathbf{r})$, of *a*, BeO and *b*, HeBeO. Dashed contours, regions of increased charge concentration (relative to the unbound atoms); solid contours, charge depletion.

In contrast to their isoelectronic relative HeBeO, neither HeBN nor HeLiF is bound. This is because the Lewis acid (σ-acceptor) capability of BeO, BN and LiF is dependent on the effective charge at the σ-acceptor site (Be, B and Li, respectively). That effective charge is determined by both the nuclear charge of the σ-acceptor-site atom and the electronegativities of the atoms of the diatomic. Thus, the effective charge at lithium in LiF cannot be greater than +1 whereas, as the calculations show, the beryllium in BeO approximates to Be^{2+}. But the boron in BN has a lower effective charge than the beryllium in BeO because of the higher electronegativity of boron relative to Be and the lower electronegativity of nitrogen relative to oxygen.

Preparation and identification of the HeBeO molecule presents an important challenge to the experimentalist. Although bound, the large increase in entropy associated with the dissociation to He and BeO means that thermodynamic stability