This Focus issue brings together the cornucopia of strategies that pathogens and tumors utilize to avoid immune recognition. Here Rodney Phillips discusses some general principles that emerge from this analysis.

Immunology taught by Darwin

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Nothing in biology makes sense except in the light of evolution.
Theodisius Dobzhansky

We are still struggling to understand the implications of Charles Darwin's monumental achievements. The most profound biological insight of the 19th century created a framework for understanding variation in organisms that has yet to be fully realized. Before Darwin, biological difference was arrayed before us in endless, unstructured confusion. Darwin made this variation a highly legitimate subject for analysis by recorded observation but also by experimental science. Nearly 150 years after the publication of the *Origin of the Species*[1], we are still laboring to understand evolution and selection in many biological systems. Twentieth century science gave us tools, which, for the first time, allowed us to look directly at the biochemistry that enables genetic variation[2]. But we are still often unable to explain the mechanisms that underlie clashes between one organism and another.

Darwin envisaged selection operating over millennia, with a tempo dictated by brutish struggles in a very competitive world. When complex organisms like humans come under threat from foreign microorganisms and parasites or from within by malignant cellular change, a Darwinian framework is essential if we are to understand these much-accelerated evolutionary encounters. The rapid growth of microorganisms and the huge potential for mutation and recombination in RNA genomes can produce high-speed versions of Darwin's original conception. If we define immunity as the organism's sum capacity to resist attack, then with a tempo that can be as short as days or as long as decades, this defensive resistance constitutes a formidable selection force on malignant cells or pathogenic agents. Immune selection determines the repertoire of bacteria that colonize the skin, the time it takes to die from HIV infection and contributes to a tumor-free youth in humans.

When assaulted by a new foreign organism, the immune system produces three basic outcomes: early, complete expulsion of the foreigner; overwhelming infection with failure of control; or persistence with potential long-term carriage or induction of disease. It is this last scenario that allows the immune response to become a sustained selection force, although the dynamics of short clashes can still select for evasive properties in some highly mutable pathogens[3].

Burnet, inspired by Lewis Thomas, thought the adaptive immune response was involved in continuous "surveillance" for malignant transformation in cells[4]. As reviewed by Schreiber and colleagues in this issue of *Nature Immunology*, the concept was developed, refuted and has now had a renaissance. A key prediction of the immune surveillance hypothesis is that breakthrough tumors will have immune-evasion properties. Like microorganisms, tumors require adaptations that allow evasion of the immune response before they can grow in an immunocompetent host.

What are the mechanisms of immune escape? The reviews contained in this Focus issue of *Nature Immunology* describe immune-escape by pathogens and tumors. Some principles emerge from this extensive catalog.

Dormancy

There is no better way to hide from immunity than to minimize antigen expression. The integrated DNA form of a retrovirus, the minimal protein production of a herpesvirus and the quiescent forms of mycobacteria all have this capacity. But the strategy has limits. Although some cells harboring HIV proviruses may indeed express no viral proteins, others become highly activated and viral proteins are readily processed for presentation to $CD8^+$ killer T lymphocytes[5]. Successful immune evasion under these selective conditions then depends on other strategies such as antigenic variation[6]. Thus microorganisms can have an array of immune-evasion strategies each designed for specific settings.

Sequestration

Cellular and humoral forms of immunity are pervasive, although the "surveillance" does have blind spots. Some organisms have evolved to occupy special niches where immunity may not penetrate or is frustrated. Malarial parasites have an asexual phase within red blood cells. These complex organisms pay the price of adaptation to life in a biochemically simple environment; the lack of major histocompatibility complex (MHC) class I molecules on the red blood cell surface means that the presence of the plasmodia will not be announced to the immune system in the form of antigenic peptides bound to MHC. Other organisms, such as *Mycobacterium tuberculosis*, also live within cells and show very low turnover. But how this is achieved is not understood. Genome comparisons between *M. tuberculosis* and *M. leprae* provide a clue[7]. Less than half the genome of *M. leprae* contains functional genes, but there are many pseudogenes with intact counterparts in *M. tuberculosis*. Gene deletion and decay have eliminated many important metabolic pathways. The potential evolutionary advantage of this is apparent: *M. leprae* and its metabolically "crippled" state has a doubling time of approximately 14 days compared to a faster rate in *M. tuberculosis*. This provides some hint as to why *M. leprae* has such a remarkably persistent, indolent lifestyle.

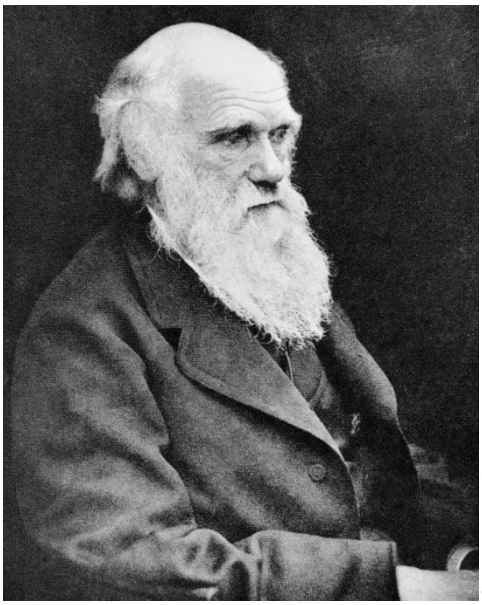
Gene loss in certain organisms seems to have led to an enhanced capacity for highly specialized sequestration. *Salmonella enterica* Typhi has 145 fewer functional genes than its relative *S. enterica* Typhimurium. Several of the lost genes are responsible for bacterial attachment; thus, loss of a specific function may enhance the ability of a microorganism to colonize sites such as the gall bladder, where

persistence is favored. Many pathogens appear to enhance virulence by the paradox of gene reduction[8]. This process of gene loss may produce an irreversible spiral if DNA cannot be reacquired to complement the heavily diminished genome of such organisms ("Muller's ratchet") and so lead to an evolutionary dead end[9]. This sort of evolutionary decline reflects the "blindness" of adaptation.

Small changes in viral genomes, which alter receptor usage, may also enhance the capacity for survival. HIV envelope polymorphisms, which allow binding to dendritic cells, may allow these cells to be used as a "Trojan horse", ferrying the virus away from mucosal surfaces to lymph nodes[10].

Failure of antigen display

Burnet's persuasive arguments in favor of immune surveillance were predicated on the existence of a cellular immune response capable of sensing dangerous antigenic change within cells, wherever they might lie in the body[4]. The vigor of allograft rejection was viewed by Thomas as a purely artificial and contrived demonstration that histocompatibility was a safety mechanism to prevent transmission of cancer[11]. How could these difficult concepts be reconciled? And what is the evolutionary role of the adaptive immune system?



Engraving of Charles Robert Darwin (1809–1882).

Some of this uncertainty was clarified when Zinkernagel and Doherty defined the genetic significance of MHC class I[12]. These highly polymorphic molecules dictated or "restricted" the response of T cells to foreign antigen. Subsequently, Townsend, in a *tour de force* of experimental biology, showed unequivocally that MHC class I acted as a molecular clasp, displaying on the cell surface short peptides derived from viruses[13,14]. We now know that the vast majority of MHC class I molecules display peptides derived from self, that is, normal cellular proteins. Here was a major clue concerning how the mutant, defective or parasitized cells could alert the policing lymphocytes of the cellular immune response. When a virus harnesses intracellular machinery, a fraction of viral protein is diverted into an active and elaborate process, which loads MHC class I with peptide. Unless this pathway of "processing and presentation" is blocked or subverted, the killer lymphocyte response will inevitably sense intracellular infection and destroy the parasitized cell.

In cancer, a more speculative extrapolation was that dangerous mutation would also be signaled to the killer T cells when altered self-peptides arrived at the malignant cell surface bound to MHC class I.

Reviews published in this issue of *Nature Immunology* provide overwhelming evidence that viruses, which have paramount success in establishing chronic infection, have evolved remarkably ingenious ways of subverting almost every part of the biochemistry of the MHC class I pathway. The mechanism may be as simple as a protein sequence rendered indigestible by the peptide-liberating proteasome, as with Epstein-Barr virus proteins, or the acquisition of proteins that interfere with antigen processing at several levels, as with cytomegalovirus (CMV). Indeed, human CMV represents amongst the best circumstantial evidence for the coevolution of pathogen and host[15].

Immune-surveillance theories imply that cancer cells might carry phenotypic evidence of immune evasion. In this issue of *Nature Immunology*, Khong and Restifo review the impressive evidence that tumor cells carry HLA class I deletions, which can arise through many mechanisms. They summarize the mounting, if not overwhelming, evidence that encounters with tumors bearing Fas can prove fatal to anticancer T cells. Evolutionary reasoning would argue that the acquisition of anti-immune cell weapons implies that T lymphocytes are a significant selection force in at least some cancers.

The reduction of MHC class I density on cells is detected by a specialized class of lymphocytes with the capacity to lyse these cells directly. These natural killer (NK) cells provide antiviral and antitumor defense. Successful outgrowth of virus or tumor may necessitate ways of subverting this cell population too. Strominger and colleagues review the mechanisms of escape from NK cell surveillance.

Antigenic variation

Enormous genetic variation is the hallmark of many successful pathogens. Variation is also characteristic of bacteria such as *Staphylococcus aureus*, which cause asymptomatic carriage as well as disease[16]. High-throughput sequencing has begun to reveal how variable many microorganisms really are. When subjected to intensive sequencing, some pathogens—such as HIV and meningococcus—are so variable that new analytic tools are required to make some sense of their genetic complexity. One masterly survey of epidemic meningitis identified nine "genoclouds" (a frequent genotype plus its epidemiologically associated descendents) that have been responsible for three pandemic waves of disease since the 1960s[17]. The authors argue convincingly that loci under positive selection are highly antigenic and represent immune escape from herd immunity[17]. Many of the variants identified are not fixed over the long term, presumably because of diminished fitness as compared with the parental genotype[17].

The concept that immunity may shape the detectable diversity in complex genotypes is not new, but in the HIV field this has been highly controversial. One group has argued consistently that the cellular immune response (or indeed any natural selection force) does not positively select for HIV variants[18]. In this issue of *Nature Immunology*, Yewdell and Hill review recent evidence for retroviral immune selection and emphasize the definitive studies that have described positive selection for SIV antigenic variants as exerted by cytolytic T lymphocytes (CTLs)[19]. Another study provides strong genetic evidence that HLA class I is closely linked to HIV polymorphism in a large cohort[20]. After 12 years of controversy[6], the current balance of evidence favors the view that CTLs exert positive selection and so shape the HIV quasispecies in individuals and perhaps also in populations[20].

Vaccine-induced immunity has been linked with antigenic polymorphism in bacteria. In 1953 mass pertussis vaccination was introduced in the Netherlands, but the disease has remained endemic with

occasional outbreaks, including a remarkable increase since 1996. Sequence comparisons between the vaccine and recent isolates showed nonsynonymous mutations in a key antigen-coding allele; this finding has been put forward as an explanation for poor vaccine performance in the face of the new prevailing strains[21]. There is similar evidence for meningococcal vaccine failure[22] and for the vaccine-induced $CD8^+$ response in the SIV model[23].

Antigenic variation represents the most severe barrier to lasting successful preventative vaccination in many infectious diseases, including, *par excellence*, HIV[24].

Conclusion

Warm-blooded, long-lived vertebrate hosts such as humans have survived because of the extraordinary capacity of the immune response to repel potentially harmful microorganisms. It is clear that pathogens (and tumors) have highly evolved ways of subverting host immunity and that although shaped (or "sculpted") by the immune response, the dangerous outgrowth often prevails. In this century as we reflect upon the achievements of Watson and Crick, Burnet, Medawar, Zinkernagel and Doherty, Townsend and the rest, it must humble modern biologists to think how far we have to go before we master the challenges posed by Charles Darwin. Indeed we are still learning to be his contemporaries.

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