

like an unfortunate event for tumor cells. After all, tryptophan is one of the essential amino acids. In fact, older literature described interferon- γ -induced IDO expression in tumor cell lines⁵, but interpreted IDO expression as a harbinger of tumor cell death. However, recent studies suggest that T cells may be particularly sensitive to the negative effects of low tryptophan levels combined with tryptophan metabolite accumulation. Under these conditions, T cells fail to proliferate, and undergo cell cycle arrest in mid-G1 phase^{6,7}. These data suggest that IDO expression by tumor cells could stall surrounding T cells by creating a local tryptophan sink, while tumor cell proliferation continues relatively unimpaired.

Is IDO expression by human tumors a direct consequence of escape from T-cell attack? If one takes the darwinian perspective of cancer progression, the answer might be no. The individual cells within a tumor mass can be considered as units of selection, and cells that proliferate faster or survive longer will become increasingly prevalent. The available data suggest that IDO expression only hampers T-cell attack in a very indirect manner, by reducing the size of the local T-cell pool. Indeed, IDO expression has no effect on the sensitivity of tumor cells to T-cell lysis, at least *in vitro*⁴. Thus, it seems difficult to imagine how IDO expression would selectively benefit IDO-

positive tumor cells compared to their IDO-negative neighbors; in this case, the frequency of IDO-positive cells would not be predicted to rise over time (Fig. 1). As selection aficionados may notice, this argument bears similarity to the view that it is unlikely that rare tumor cells with a metastatic phenotype will become increasingly present within a growing primary tumor mass⁸.

If IDO expression by tumor cells is not caused by T-cell selection, what do tumors do with IDO? We see three possibilities. First, IDO expression might lead to a selective advantage for rare tumor cells through an effect that is unrelated to its antiproliferative effect on T cells. The fact that the observed effects of IDO are highly diverse⁹ makes this a more than theoretical possibility. Second, IDO could be induced by environmental factors. Third, IDO may be expressed at an early stage in a large fraction of growing tumors, for instance through coregulation with an oncogenic pathway.

Two lines of inquiry may help clarify some of these issues. A selective advantage of IDO expression and the possible role of T cells in this selection could be easily revealed by following the outgrowth of IDO-positive and IDO-negative tumor cells when inoculated as mixtures in the absence or presence of T-cell attack. In parallel, it would be useful to establish whether IDO is expressed in

spontaneous or chemically induced mouse tumor models. If so, it will then be straightforward to determine whether IDO expression occurs less frequently when such tumors arise in T-cell-deficient mice.

Although we may not presently understand how or whether IDO expression is selected for during human tumor growth, there is strong evidence that IDO expression can limit CTL attack. As Uyttenhove *et al.* point out, the ability to manipulate this pathway with inhibitors may make it possible to counteract the subversive effects of IDO expression by tumor cells on T-cell immunity. Until then, the same unexpected molecular mechanism that protects us during gestation may contribute to our final demise.

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Mutants escape from killer T cells, invade population

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A frenzied bout of evolution occurs every flu season, as viruses test the limits of the human immune system. Variants that can evade attack by cytotoxic T cells gain a firm foothold in the viral population even if they confer an advantage in a low percentage of human hosts. A new mathematical model examines this unusual dynamic.

Escape from immune responses is a widespread characteristic of many infectious diseases¹, including malaria, HIV and hepatitis. Antigenic escape often allows pathogens to evade clearance and establish persistent infections that eventually result in pathology. Consequences of immune escape for *in vivo* infection dynamics have been widely studied both experimentally^{2,3} and with mathematical

models^{4,5}. Equally important is the spread of escape mutants through the host population. Although much work has been done on the epidemiology of antibody escape mutants^{6–8}, little is known about the population-level impact of virus mutants that escape from cytotoxic T-lymphocyte (CTL) responses.

In a recent issue of *Proceedings of the National Academy of Sciences* (PNAS), Gog *et al.*⁹ begin to fill this gap. They present mathematical models that describe the spread of CTL escape mutants through a host population in the context of influenza A virus infection. They use this model to explain the rapid invasion of CTL escape mutants that is

observed in epidemiological data sets. The mutant completely replaces the wild-type virus, a phenomenon called fixation. Rapid fixation of CTL escape mutants is brought about by a combination of enhanced low-level persistence of the viral infection during interepidemic periods, and by a founder effect in which a small number of founding cases initiates the epidemics.

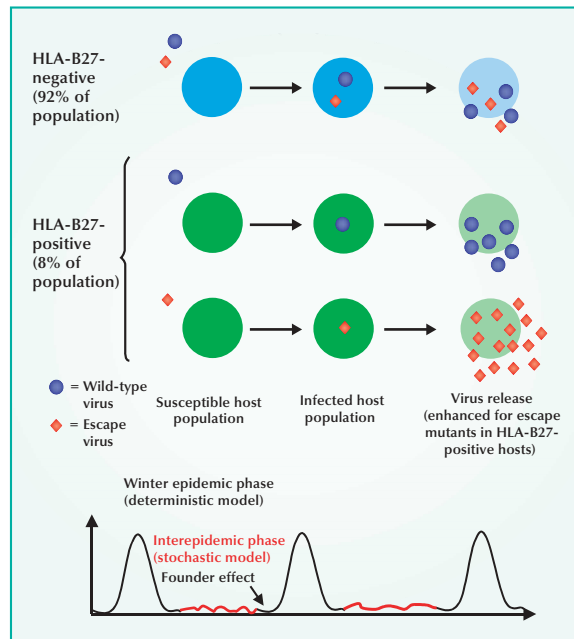
The relative influence of the two major immune effector mechanisms, CTL and antibody responses, in viral infections is debated and varies with different types of infections. With the flu, antibody responses take the dominant role in protecting against reinfection.

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Figure 1 Gog *et al.* present a mathematical model for the dynamics of influenza infection and viral escape from an HLA-B27-restricted CTL response.

(a) The majority of hosts are HLA-B27-negative, and in this context the CTL escape mutant does not have a fitness advantage. Eight percent of the hosts are HLA-B27-positive. In these hosts, infection with a mutant virus results in delayed clearance compared with wild-type infection. This can result in either increased infectivity or increased length of infection. The net effect of both is an increase in the basic reproductive ratio of the escape mutant. (b) To explain the rapid fixation of CTL escape mutants in epidemiological data sets, the basic model outlined above must be coupled with a stochastic model that includes interseason dynamics, during

which influenza persists at low numbers. During this interseason phase, the CTL escape mutant can have a key survival advantage. This, together with founder effects at the start of an epidemic, can account for the rapid fixation of CTL escape mutants, even if the fitness advantage of the escape mutant is relatively small and the advantage occurs in only 8% of the host population.



tion. CTLs might have a limited protective role in the context of reinfection, but they are thought to be primarily important for virus clearance. Reduced CTL-mediated activity can result in delayed resolution of infection¹⁰. The emergence of virus variants that escape from antibody responses is a central characteristic of influenza epidemiology and poses significant challenges for the design of effective vaccines^{6–8}.

The epidemiology of CTL escape brings with it additional complexity: CTLs recognize their epitopes in conjunction with major histocompatibility complex (MHC) molecules, which are highly polymorphic. In other words, an epitope that is recognized by some hosts is not recognized by others, so an escape mutant would only be beneficial in a fraction of the host population.

Building on previously established epidemiological frameworks, Gog *et al.*⁹ try to explain a puzzling observation in epidemiological data sets from the Netherlands and other countries: when CTL escape mutations occur, they can reach fixation rapidly. An example is the loss of a CTL epitope on the influenza A nucleoprotein that is associated with the *HLA-B27* MHC allele, which is present in only 8% of white individuals.

How can the escape mutation reach rapid fixation if it only confers an advantage to the virus in 8% of its hosts? Consistent with experimental data, the models assume that

CTL escape can result in delayed clearance. This may result either in increased infectiousness or in an increased infection length; both enhance the reproductive potential of the pathogen.

The models (Fig. 1) take into account both the within season (winter) dynamics of an epidemic and the low-level, stochastic persistence of the flu during interepidemic periods. The deterministic dynamics occurring during the winter epidemic are not sufficient to account for the invasion of CTL escape mutants in the relevant time frame, unless the fitness advantage of the escape mutant is unreasonably large. On the other hand, the escape mutation confers a key survival advantage to the virus during the stochastic phase of low-level persistence between epidemics; this can contribute to rapid fixation. In addition, the effect can be amplified if an epidemic is started with a small number of founding cases. Rapid fixation occurs even if the advantage of escape relative to wild type is slight, and even if the advantage only applies to 8% of the host population.

This study is a first step at using mathematical models to explore the population-level impact of escape from T-cell responses and MHC restriction, and provides an elegant explanation for the rapid fixation of influenza CTL escape mutants in epidemiological data sets. However, many questions remain regarding the impact of CTL escape at the popula-

tion level, even in the specific case of influenza virus infection. What are the exact effects of CTL escape on the nature of the immune response and the ability of the virus to spread through the host population? This question lies at the core of model assumptions.

Although data support the notion that CTL escape can result in delayed clearance, experiments with influenza-infected mice have shown that the effects of CTL escape on host immunity can be complicated and counterintuitive¹⁰. In these experiments, researchers constructed virus variants that had point mutations in the two most prominent CTL epitopes, such that they could no longer be recognized.

In accordance with model assumptions, inactivation of a single epitope resulted in delayed clearance and reduced survival compared with wild-type viruses. Inactivation of both epitopes, however, resulted in a level of protection that was comparable to that of wild-type viruses. Further experiments offered an explanation for this surprising observation: reduced CTL-mediated activity correlated with a compensatory increase in neutralizing antibody responses as a result of stronger antigenic stimulation, which in turn facilitated protection. Previous mathematical studies of immune responses against persistent infections suggested similar trends^{11,12}. Antibody and CTL responses are stimulated by the same viral population and are therefore in competition with each other. Escape from one response can lead to an increase in the other.

More detailed knowledge of the immunological consequences of CTL escape will be required to further develop our understanding of the spread of such mutants through the host population. The study by Gog *et al.*⁹ provides a new framework upon which to build such explorations.

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