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HYPOTHESIS

How does Cytopathicity Affect the Production of Neutralizing Antibody?

C. KEŞMIR* & R. J. DE BOER†

**Department of Biochemistry & Nutrition, Technical University of Denmark, Lyngby, Denmark; and †Theoretical Biology Group, Utrecht University, Utrecht, The Netherlands*

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Cytopathic viruses evoke an earlier neutralizing antibody (nAb) response than noncytopathic viruses do. This was previously explained by the elimination of infected B cells by the cytotoxic T cells (CTLs), which predominate during infections with noncytopathic viruses. Using a simple mathematical model we provide a much simpler explanation for this difference in the kinetics of neutralizing antibody production. The analysis of the model shows that the delay in nAb production during infections with noncytopathic viruses is a simple consequence of the cytopathic effect alone: noncytopathic viruses infect a larger fraction of nAb-producing B cells and as a result nAb response is delayed. Extending the model with CTLs, we find that a major effect of CTLs is to limit the antigenic stimulus of the nAb-producing B cells. Thus, by reducing the proliferation rate of nAb-producing B cells, CTLs further delay the production of neutralizing antibodies.

Can Keşmir, Department of Biochemistry & Nutrition, Technical University of Denmark, Bld. 224, DK-2800 Lyngby, Denmark

A number of studies have suggested that the induction of neutralizing antibody (nAb) responses is critical for virus elimination and for protective immunity [1–7]. A decrease in the infection rate is ascribed to the fact that nAb efficiently blocks the receptors mediating infection. In infections with cytopathic viruses, nAbs are generated within 6–14 days, whereas with some viruses, for example human immunodeficiency virus (HIV) [8–10], hepatitis B virus (HBV) [11] and lymphocytic choriomeningitis virus (LCMV) [1], they are not generated until 50–150 days have elapsed. Both LCMV and HBV are noncytopathic viruses; thus these observations suggest a correlation between the degree of cytopathic effect and the speed of nAb production. Non-neutralizing antibodies appear very early during LCMV and HBV infections, therefore the delay in the nAb response cannot be explained by reduced antigenic stimulus [1]. However, nAb-producing B cells do become infected in LCMV and HBV infections, most probably because neutralizing surface immunoglobulin might serve as a receptor for the infection [2, 11]. Thus a possible cause of the delay could be the elimination of these infected B cells by cytotoxic T cells (CTLs) [1, 2, 11]. There are, however, two open questions in this scenario. First, in the case of a noncytopathic virus, why would the CTLs stop killing infected nAb producers after 50–150 days? Second, how do B cells in general resist infection by cytopathic viruses?

Using a simple mathematical model we investigate whether it is possible to verify the correlation between the kinetics of nAb production and the degree of cytopathic effect of the infecting virus. The model assumptions are inferred from experimental data on the nAb response to LCMV [1, 2], but should also apply to other viral infections. Both infected and noninfected hybridomas secreting nAb are found *in vivo* [2]. Thus we assume that a nAb-producing B cell can either become infected, or become activated and proliferate. In a germinal centre, for example, nAb-producing B cells can receive activation signals from follicular dendritic cells without being infected, but in the same environment free virions can be infectious. We can write the following ordinary differential equations for LCMV-specific nAb-producing B cells:

$$\frac{dB}{dt} = pB - \beta BI - \delta B, \tag{1}$$

$$\frac{dI}{dt} = \beta BI - (\delta + \alpha)I, \tag{2}$$

where B and I are noninfected and infected B cells, respectively.

The parameter δ is the death rate of B cells, and α is the degree of cytopathic effect of the virus ($\alpha = 0$ represents a noncytopathic virus). The infection rate is proportional to the density of infected B cells I, i.e. noninfected B cells become infected at a rate βBI. This seems a realistic assumption in a germinal centre where B cells are the major cell type. The parameter p is the maximum rate of proliferation of B cells. In this model we assume that the antigen concentration is not limiting for B-cell proliferation or antibody production. This assumption will be relaxed later (see below). The steady state of the system is found by solving $dB/dt = dI/dt = 0$, i.e.

$$\hat{B} = \frac{\delta + \alpha}{\beta}, \quad \hat{I} = \frac{p - \delta}{\beta}, \text{ and } B_T = \hat{B} + \hat{I} = \frac{p + \alpha}{\beta}. \qquad (3)$$

The steady-state expressions in equation 3 suggest that both the total number of B cells, B_T, and number of uninfected B cells, $\hat{B}$, increase linearly with the cytopathic effect, α, of the virus. If we assume that the rate of antibody production is proportional to uninfected B cells (or, also, to total B cells), the model predicts that cytopathic viruses evolve a stronger nAb response, which is in good agreement with the data [12]. Thus low cytopathic effect alone explains the late nAb response in noncytopathic viruses. We explain this result as follows: Long-lived infected B cells allow for more secondary infections than do short-lived infected cells. Thus, if as a result of low cytopathic effect, infected B cells remain long-lived, the LCMV infection will spread and affect a larger fraction of the B cells. Because infection might reduce the capacity of a cell to expand, low cytopathic effect implies reduced clonal expansion, and hence slower and later nAb production. Because non-neutralizing antibody producers do not become infected [2], their clonal expansion is not influenced by the cytopathic effect.

So far we have not included the effect of a CTL response in the model. However, it was demonstrated that $CD8^+$ T-cell depletion reduces the delay in neutralizing anti-LCMV antibody production [1, 2]. We consider two effects of a CTL response on the B-cell dynamics. First, activated $CD8^+$ T cells kill infected nAb-producing B cells [2], i.e. they decrease I. Second, by decreasing I, they decrease the antigen concentration and limit antigenic stimulus of the nAb-producing B cells [1, 11, 13]. This was demonstrated by the increased numbers of LCMV-specific B cells (both neutralizing and non-neutralizing) in anti-CD8-treated animals [2]. To incorporate the dependence of B-cell proliferation on the antigen availability (I), we update equation 1 to

$$\frac{dB}{dt} = \gamma B - \beta BI - \delta B, \qquad (4)$$

where γ is a competitive proliferation function,

$$\gamma = \frac{pI}{s + B}, \qquad (5)$$

derived previously [14]. This proliferation function γ allows for competitive regulation of B-cell proliferation under continuous antigenic stimulus; the *per capita* rate of proliferation decreases with increasing number of noninfected B cells. By introducing γ we ensure that B-cell proliferation is limited both by the antigen availability and by the B-cell population size. Solving $dB/dt = 0$, one can express the steady-state expression of B in terms of the infected cells I,

$$\hat{B} = \frac{\hat{I}(p - s\beta) - s\delta}{\delta + \hat{I}\beta}, \qquad (6)$$

which is an increasing function of I, i.e., $\partial\hat{B}/\partial\hat{I} = (\delta p)/[(\delta + \beta\hat{I})^2] > 0$. Thus, if $CD8^+$ T-cell depletion increases number of infected B cells [2], the model predicts that the number of noninfected nAb-producing B cells increases, which would evoke a faster nAb response. This simple explanation differs from a previous suggestion arguing that the speeding-up of nAb response in anti-CD8-treated animals is due to reduced elimination of infected B cells, as these cells can also produce nAb, albeit 2–3-fold less than the virus-free cells [2]. We here argue that the speeding up can also be due to the enhanced proliferation of noninfected B cells as a result of a continuous antigenic stimulus.

The kinetics of non-neutralizing antibodies should not be influenced by the cytopathic effect of the pathogen, because non-neutralizing antibody-producing B cells are not infected with LCMV [2]. Likewise, $CD8^+$ T-cell depletion fails to affect non-neutralizing antibody production [2]. Because the primary non-neutralizing antibody production is already very rapid, we would argue that it is not accelerated by $CD8^+$ T-cell depletion.

To summarize, the simple models we have developed yield two main results. First, the degree of cytopathic effect alone can explain the kinetics of nAb production. Second, $CD8^+$ T cells have a 2-fold effect on the nAb dynamics: apart from killing infected B cells [2], we conjecture that $CD8^+$ T cells also suppress the proliferation of the nAb-producing B cells by limiting the antigenic stimulus. Although these results are based on LCMV data, they should remain valid for any virus with capability of infecting nAb-producing B cells.

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