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Assessing the Mechanisms That Give Rise to Autoimmunity

I read with great interest the report by Kouskoff *et al.* (1), in which the authors used an immunoglobulin M (IgM) non-switchable B cell transgenic (Tg) mouse to show that the B cells could be made to react against a phage exhibiting a cross-reactive antigenic determinant of 20 amino acids in a polymeric form. This antigen was bound by the Tg antibody receptor and induced antibodies as determined in an enzyme-linked immunosorbent assay (ELISA), which probably assessed low-avidity antibodies (though this was not measured or stated). The favored interpretation of Kouskoff *et al.* was that infectious agents can break B cell tolerance by expressing repetitive epitopes that mimic self.

Surprisingly, Kouskoff *et al.* did not discuss earlier experiments and interpretations in which we showed that an antigen Tg mouse expressing the glycoprotein of vesicular stomatitis virus (VSV-G), and with no manipulation of the immune system, was unresponsive against VSV-G if the Tg mice were immunized with the antigen in purified form, even in complete Freund's adjuvant (2, 3). If the same mice were exposed either to replicating VSV or formaldehyde-fixed VSV, however, a prompt, T-independent type 1 (TI-1) IgM B cell response was induced. We concluded that the Tg mice could not develop an IgM or IgG response against the Tg self-glycoprotein in an oligomeric or monomeric form, and that T help was limiting because these Tg mice were tolerant at the level of T help (4). In contrast, the polymeric form of VSV-G on the viral envelope induced B cells to produce IgM in a completely TI-1-dependent fashion in the absence of polyclonal activation (5) by cross-linking Ig receptors (6, 7). The fact that neutralization of virus requires an affinity of greater than $5 \times 10^7 \text{ M}^{-1}$ (8) suggests the considerable affinity of these neutralizing antibodies. This IgM response also switched to IgG because the virus particle included nuclear protein and matrix protein that in turn induced new T cell help that could be recognized in a linked fashion (2, 3).

The conclusion of other researchers and of ourselves (5, 6), therefore, was that B cells in general are not tolerant and can switch. B cells do not react and do not get induced in absence of T cell help, however, if the Ig-surface receptors are not optimally cross-linked by paracrystalline rigidly polymeric repetitive identical determinants, such as are exposed on viral envelopes, on bacterial outer membranes, or on classical parasites (9, 10).

Fehr *et al.* (11) took this evidence one step further by comparing auto-antibody responses against syngeneic IgM or allotypically marked IgG antibodies in mice (the IgG allotype carries a serologically defined difference that could be analyzed by ELISA). Fehr *et al.* immunized mice with the neutralizing anti-VSV-G antibody alone, with VSV alone, or with preformed complexes between VSV and the neutralizing monoclonal IgM or allotypically marked IgG antibody (11). In the first two examples, no anti-antibodies were formed that could be measured, whereas, in the third case, antibodies against IgM or anti-allotypic antibodies were promptly formed within one or two immunizations with the complexes. This further illustrated and supported the simple notion that, if self-antigens are exhibited in a highly repetitive form—as is the case for these antibodies made repetitive because of the repetitive matrix of the VSV-G on the viral envelope—then B cells react and are promptly induced even against self-IgM or self-IgG proteins in serum.

We concluded (8–11) that against a sufficient dose of highly repetitive, identical polymeric determinants, B cells are not tolerant and react in a TI-1 fashion without an obvious polyclonal activator—particularly if the determinants are spaced by about 8 to 10 nm (equivalent to the distance between the two recognition sites on the immunoglobulin). In contrast, against monomeric antigens, B cells strictly and exclusively react in a T cell–dependent and linked fashion; therefore, autoantibody responses against monomeric or oligomeric self-antigens, as they become accessible to B cells in the blood of the intact host, are not induced because the obligatorily necessary T cell help is not available. Instead of the still unproven mimicry hypothesis, the results outlined above suggest that, besides antigen dose, antigen patterns plus absence or presence of T cell help play the principal roles in regulating B cell responsiveness (5, 6, 10).

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Response: We regret any omissions in our report (1), particularly our failure to cite the paper by Bachmann *et al.* (2), which should have been included among the citations in reference 4 of (1). We must, however, respectfully disagree with Zinkernagel on certain other points. Zinkernagel argues that immune tolerance does not occur at the B cell level but exclusively through other forms of regulation. Although this view may be consistent with his data, it cannot be generalized without denying a large body of direct evidence to the contrary (3–16). Figure 1 of Kouskoff *et al.* (1) showed two obvious consequences of self-tolerance: clonal elimination leading to reduced precursor frequency of reactive cells, and reduced antibody response upon challenge with a cross-reactive microbe. These results remind us that tolerance, like immunity, is a quantitative phenomenon.

The role of antigen multivalency in our study is unknown. The foreign antigen that we used has only five or fewer epitopes, which are not believed to be paracrystalline or rigid, but displayed on a flexible linker.

With respect to the use of antibody Tg mice, we acknowledge that, by design, they distort B cell repertoire and heavy-chain class switching. They have the compensatory advantage, however, of allowing tracking of antigen-specific cells. This permitted us to verify that the responding B cells were not ignorant of self because of antigen inaccessibility or immaturity. Nor did they carry a different antigen receptor from those of B cells developing in the absence of self-antigen; instead, upon immunization, they were deviated from their ongoing tolerogenic program. Because such determinations are difficult or impossible to make in a polyclonal model—and, indeed, were not made in the studies cited by Zinkernagel—our results provide novel mechanistic insight that complements other types of studies.

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