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Christopher C. Goodnow

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Nossal and Pike 1975: A Turning Point in the Effort to Define Self-Tolerance Mechanisms

Christopher C. Goodnow[1]

The 1975 publication by Gus Nossal and Bev Pike (1), reprinted as a Pillars of Immunology article in this issue of *The Journal of Immunology*, reads as an elegant and compelling set of experiments viewed through the "retrospectroscope." However, in 1975, and indeed for the next 13 years, the results of these experiments and many that followed them were not embraced widely by the field at large. Therefore, the article is significant today both as a conceptual and experimental turning point in tolerance research and because it illustrates the problems we face in science when our experimental tools do not quite allow us to overcome the fickleness of our belief systems.

The hypothesis articulated in the study generates little controversy today; unlike mature lymphocytes, immature lymphocytes in primary lymphoid organs are somehow "wired up" to favor a tolerance response over an immunity response. To test whether this was or was not the case, the team took advantage of the very recent finding at the time that surface Ig-positive small B cells are constantly differentiating from dividing progenitor cells in the bone marrow of adult mice and that this step in differentiation could proceed in short-term tissue cultures of bone marrow.

Into these cultures of nascent B cells Nossal and Pike added an Ag: one of two haptens, DNP or 4-hydroxy-3-iodo-5-nitrophenylacetate (NIP),[2] coupled to the protein human γ-globulin (HGG). HGG had become a favorite protein carrier for in vivo tolerance research in the 1960s and 1970s; it was readily available in pure, soluble form in large quantities, was easily conjugated with haptens, and had a long pharmacological half-life in vivo. Additionally, it had the interesting property that it was tolerogenic when given to mice in a form that was free of aggregates but became immunogenic if given in aggregated form or if contaminated with a little LPS endotoxin (2–4).

After 1–3 days, Nossal and Pike (1) harvested the cultured immature B cells and tested their capacity to mount an Ab response to the same hapten delivered as an immunogenic conjugate with a different protein carrier, polymerized bacterial flagellin (POL). We know now that the immunogenicity of POL shares a common mechanism with that of LPS; both are stimulatory ligands for related TLRs (TLR5 and TLR4, respectively) and through these receptors promote B cell proliferation and plasma cell differentiation without the need for T cell help. Nossal and Pike transferred the cultured B cells into irradiated lymphopenic mice, challenged the mice with DNP-POL, and measured the number of DNP-specific plasma cells by the Cunningham plaque assay.

The results speak for themselves. In vitro exposure of nascent bone marrow B cells to DNP-HGG markedly diminished the DNP-POL plasma cell response but had no effect on the NIP-POL plasma cell response, and vice versa. By contrast, the same in vitro treatment of mature B cells from the spleen had no effect on their subsequent capacity to mount a DNP-POL plasma cell response. T cells were not required for tolerizing the bone marrow cells and the effect was cell autonomous because mixing tolerized and control cells did not suppress the latter's response, ruling out suppressor T cells, a popular cell-to-cell mechanism at the time.

The proposed mechanism of "clonal abortion" of nascent lymphocytes articulated by Nossal and Pike reawakened a version of Frank Macfarlane Burnet's clonal deletion hypothesis that had emerged at the end of the 1950s when Nossal was a Ph.D. student and Joshua Lederberg was a sabbatical professor in Burnet's Melbourne laboratory (5). The concept also illuminated earlier discoveries between 1970 and 1972 from Max Cooper's group, which discovered that few or no B cells developed in chickens or mice treated with Ab against IgM beginning in development before B cells had formed (6). Cooper's group had focused primarily on these results as compelling evidence that all B cells begin as IgM^+ and then switch to IgG, IgA, etc. Nevertheless, they had noted briefly that the result might reflect a physiological mechanism for self-tolerance: "Acting at an early stage of differentiation, anti-mu antibody might inactivate or eliminate. . . cells at the time that they begin to express IgM surface antibody. . . . This concept is analogous to the elimination of 'forbidden clones' recognizing self-antigens" (6).

Stimulated by the data and concepts in Nossal and Pike's 1975 article (1), Max Cooper's colleague Martin Raff and Emil Unanue's laboratory independently followed up that same year with experiments culturing immature bone marrow cells with anti-IgM that engaged all nascent B cells (7, 8). This approach reinforced the clonal abortion concept by showing that immature B cells, unlike mature splenic B cells, underwent irreversible modulation of cell surface IgM. The clonal abortion concept was extended further with sophisticated tissue culture methods used by Normal Klinman's group in 1976 (9) and by an extensive series of experiments by Nossal, Klinman, and other groups through to the early 1980s (10).

[1] Address correspondence and reprint requests to Dr. Christopher C. Goodnow, The Australian National University, Immunology and Genetics Division, Australian Phenomics Facility, John Curtin School of Medical Research, Phenomics Building 117, Canberra, Australian Capital Territory, 0200 Australia. E-mail address: chris.goodnow@anu.edu.au

[2] Abbreviations used in this paper: NIP, 4-hydroxy-3-iodo-5-nitrophenylacetate; HGG, human γ-globulin; POL, polymerized bacterial flagellin.

At the time, why were such compelling results not widely embraced? Technical limitations were one factor. The experiments depended upon tissue culture systems that might not reflect the physiological response of B cells in vivo, tolerance only worked with some Ags but not others, and the actual fate of Ag-engaged B cells during tolerance could only be inferred indirectly except when the artificial stimulus of anti-IgM was used. In their subsequent experiments, Nossal's group found that many of the tolerant cells appeared not to have been aborted and introduced a new concept to explain the fate of immature lymphocytes, "clonal anergy" (10). This shift away from Burnet's simpler explanation encouraged theoreticians to argue irrelevance: why would the immune system bother to retain forbidden clones in a less responsive state? In the absence of a way to directly trace the fate of self-reactive lymphocytes in vivo, there remained much latitude for alternative views about the mechanism of self-tolerance.

With in vivo self-tolerance essentially a black box at the cellular and molecular level, popular theories had swung pendulum-like under the influence of a little data and a lot of interpretation. Clonal deletion had been a popular theory when it was new in 1959, but this baby was thrown out with the bath water to some extent by evidence in the 1960s and 1970s that some self-Ag binding cells could be found in the circulation and by the discovery that T cells recognize foreign Ags only on cells bearing self-MHC Ags. Despite a soft experimental base, newer theories of idiotype networks and I-J positive suppressor T cells enjoyed greater popularity in the 1970s and early 1980s as theoretical explanations for self-tolerance.

Advances in molecular biology and mAbs in the 1980s helped put our theoretical imaginations back in the armchair and out of the laboratory, evaporating I-J and enabling direct visualization of self-reactive B and T cell fate in vivo. In 1987, the development by Kappler and Marrack of Abs to individual TCR V-β elements revealed that nascent T cells recognizing self-superantigens indeed are clonally aborted within the thymus (11). This was quickly followed by direct demonstration of clonal deletion and clonal anergy in T and B lymphocytes recognizing conventional Ags in transgenic mice (12–15). An unanticipated third cellular mechanism for imposing tolerance in nascent lymphocytes also emerged: self-Ag-induced editing of receptor genes, where only the expression of the offending receptor is aborted (16). Which of these mechanisms actually accounts for the experimental results of Nossal and Pike is hard to say, but there is no question that the concept proved correct and focused the minds of a generation of tolerance researchers.

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