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Alone again, naturally: B cells encountering antigen without T cells

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Despite the many conceptual advances and the identification of the nature of the second, costimulatory signals, many questions remain unclear in fully understanding the mechanisms that underlie T and B cell activation versus tolerance.

With remarkable prescience, Bretscher and Cohn in 1970 postulated that the induction of humoral antibody formation to hapten-carrier antigens required the recognition of 2 signals; recognition of the hapten by B cells, and associative carrier recognition by thymus-derived T cells (1). They further postulated that paralysis of antibody formation occurred when only antigen-recognition by B cells occurred without the associative carrier recognition, or when the carrier-specific T cells were paralyzed. Over the next 47 years, we have observed an evolution of the 2-signal theory of immune activation (reviewed in (2, 3)); with Lafferty and Cunningham's clarification (1975) of the role of a MHC-expressing stimulating-cell-derived second signal, Jenkins and Schwartz's (1987) demonstration of experimentally induced T cell anergy in the absence of signal 2, Janeway's theory (in 1989) of a second signal triggered in antigen-presenting cells (APCs) by microbial products through Toll-like receptors, and Matzinger's danger hypotheses (1994) showing that APC activation is induced by host-cell damage and recognition of "danger" signals and the recognition of antigens in the absence of danger resulting in T cell tolerance. Despite the many conceptual advances and the identification of the nature of the second, costimulatory signals, many questions remain unclear in fully understanding the mechanisms that underlie T and B cell activation versus tolerance.

Consistent with the 2-signal model, the current paradigm states that B cells activated by antigen follow 1 of 3 pathways: i), they proliferate and differentiate to give rise to a productive humoral response, ii), they acquire an anergic state or iii), they are deleted. The study by Turner et al, (3) examines the consequence of antigen-recognition by B cells in the absence or presence of T cells, by focusing on the impact of antigen persistence showing a fourth possible outcome of B cells exposed transiently to antigen. Using an elegant, 2-step exposure of BCR-transgenic B cells to antigen (Figure 1) they demonstrated that transient (5 min.) antigen exposure together with antigen-specific T cell help was sufficient to induce the full development of an antibody response and memory B cells. In the presence of persistent antigen in vivo, the B cell response was enhanced, albeit only modestly. The transient

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exposure to soluble antigen was sufficient to prime the full B cell differentiation over a broad range of antigen doses and at lower antigen affinity, underscoring the sensitivity of B cells to Signal 1.

Things became more interesting when the experiment was performed in the absence of T cell help or in T cell-deficient hosts. B cells transiently exposed to antigen and then transferred in hosts that received repeated (0, 12 & 24 h post-AdT) antigen (administered i.v.) were significantly depleted by 30 hrs post AdT, compared to B cells that were not reexposed to antigen in vivo. These observations underscore the 2-signal model for B cells with exposure to persistent antigen resulting into B cell tolerance; this observation was made in the absence of T cell help. (4, 5). However, when B cells were only transiently exposed to antigen and then adoptively transferred into recipients without T cell help or into T cell-deficient hosts, B cell numbers were comparable to naïve (nonantigen-exposed) B cells maintained in vivo. These antigen-exposed B cells migrated to the T-B border and upregulated CCR7 and downregulated IgM. However, by 24 h post-AdT, B cells had migrated away from the T-B border, downregulated CCR7 and upregulated IgM. Furthermore, B cells transiently exposed to antigen- upregulated CD86 and presented antigen:I-A^b complexes that peaked at 12 h post-AdT returning to baseline by 72 h post-AdT. This process was paralleled by the loss in their ability to engage with antigen-specific T cells 24–48 h after antigen-exposure. Most intriguingly, transiently antigen-exposed B cells were fully capable of entering into a productive immune response when reexposed in vivo, 3 or 5 days later, to antigen in the presence of T cells help. These observations demonstrate that B cells exposed to antigen without T cell help are not deleted or anergic, but rather that antigen encounters are innocuous with B cells retaining their ability to respond productively upon antigen encounter (Figure 1).

There are some caveats of this experimental model that are worth highlighting: the readout of B cell fate was completely dependent on 2 BCR-Tg B cells (MD4 or HyHEL10) specific for hen or duck egg lysozyme (HEL or DEL) that were adoptively transferred in large numbers ($1.5\text{--}4.5 \times 10^7/\text{recipient}$). Moreover, T cell help was provided by AdT TCR-Tg OTII cells ($5 \times 10^5/\text{recipient}$) activated by immunization with ovalbumin emulsified in complete Freund's antigen. Amplified numbers and affinities of transgenic T and B cells may induce cell fates that differ from endogenous cells (6, 7). Furthermore, follow-up durations for key experiments were relatively short (<5 days). Indeed, it is possible that with time, transiently antigen-exposed B cells will be deleted gradually or anergized.

Despite these limitations, these observations may have relevance to transplant recipients. For example, with the ability of B cells to engage with antigens and the capacity to receive T cell help over 24–48 h improves their chances for encountering the rare antigen-specific T cell at the T-B interface. If these B cells do not receive T cell help, they can still undergo repeated rounds of antigen exposure while preserving their ability to become fully functional upon T cell help. Since immunosuppression severely curtails T cell help, the ability of pathogen-specific B cells to return to baseline while becoming fully functional once T cell help becomes available may be critical for immunosuppressed patients in developing protective immunity after immunosuppression is reduced (8).

These findings also underscore the importance of antigen persistence for B cells to become anergic or deleted. In solid organ transplant recipients, the allograft is a persistent source of antigen yet antibody-mediated rejection is 1 of the major cause of graft loss, raising the question on why B cell anergy or deletion does not occur? There are several possible explanations: for example, under conventional immunosuppression, the incomplete suppression of T cell help prevents alloreactive B cells from undergoing deletion or anergy. Alternatively, memory B cells have relaxed restimulation requirements as a result of epigenetics, expression of high affinity BCR and costimulatory molecules (9), and may therefore be more resistant to anergy and deletion compared to naïve B cells. Indeed, the frequency of memory B cells in the peripheral blood of humans has been shown to increase with age (10). Thus, additional investigations are necessary to define the susceptibility to anergy or deletion, of memory compared to naïve alloreactive B cell in the presence of a solid organ allografts. Solving the conundrum of inducing anergy or deletion of memory B cells, in addition to memory T cells, is likely to be key to achieving successful transplantation tolerance.

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Abbreviations

AdT	adoptive transfer
BCR-Tg	B cell receptor-transgenic
DEL	duck egg lysozyme
HEL	hen egg lysozyme
BCR-Tg	T cell receptor-transgenic

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Figure 1.

Fate of B cells upon transient or sustained encounter with antigen in the presence or absence of T cell help. B cells with T cell help differentiate to give rise to a productive humoral response, B cells in the presence of persistent antigen but without T cell help acquire an anergic state or are deleted, while B cells encountering antigen transiently but without T cell help return to naïve state and retain the ability to respond productively to antigen reencounter in the presence of T cell help.