gigapascals, it is hard to prevent reversion upon return to STP because of the strain generated during pressure release. Exceptions exist: stishovite SiO_2 (as mentioned), ZnO (*10*), and AlN (*11*).

In normal processing, synthesis occurs directly from the elements or free atoms, and the temperature is high enough to convert amorphous material to crystalline material. The thermodynamic phase is invariably obtained. Perhaps instead one could catalyze synthesis of metastable phases under milder conditions closer to STP. Catalysis, well developed in organic synthesis and biology, remains primitive in solid materials. Catalysis may be involved in two recently reported reactions: GaN nanocrystals with the rocksalt structure have been made in benzene solvent at 280°C and a pressure of about 50 bar, conditions far below the region of rocksalt thermodynamic stability (*12*). Also, rocksalt CdS has been reported in a reaction templated on a polyethylene oxide film (*13*). The mechanisms are not known. In a manner similar to C_{60}, perhaps belief in the metastability of the six-coordinate phases will encourage discovery of novel and practical reactions, and thus a new area of semiconductor materials.

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IMMUNOLOGY

Antigen Presentation by Memory B Cells: The Sting Is in the Tail

David Tarlinton

Immunoglobulins, or antibodies, must bind to a vast array of foreign molecules and so themselves exist in many forms. The sequence in the variable (V) region of immunoglobulin molecules varies enormously, conferring virtually unlimited capacity to bind antigen. The so-called constant (C) region comes in five different varieties—α, δ, ε, γ, and μ—providing five different isotypes [immunoglobulin A (IgA), IgD, IgE, IgG, and IgM], each of which performs a different suite of functions. Finally, there are both secreted and membrane-bound forms of the immunoglobulins. The membrane-bound immunoglobulins are critical in the generation of B cell–mediated immune responses, participating in both signal transduction and antigen processing. Now, three sets of experiments reported on pages 407, 409, and 412 of this issue (*1–3*) and one soon to appear in *EMBO Journal* (*4*) present new information on how the many membrane-bound forms of immunoglobulin control B cell function.

Upon activation by antigen, B cells follow one of two differentiation pathways: They may differentiate directly into plasma cells, which are basically antibody-secreting factories, or they may give rise to germinal centers, specialized structures within lymphoid organs. Here, successive rounds of mutation of the immunoglobulin V region genes are followed by expression of the gene products on the cell surface and selection of the cells on the basis of the affinity of these mutated immunoglobulins. Nonselected (expressing low-affinity immunoglobulin) cells die, whereas selected (with high-affinity immunoglobulin) cells go on to become plasma or memory cells. In both pathways of antigen-induced B cell differentiation, isotype switching occurs in which the C region of the immunoglobulin heavy chain changes from the joint expression of IgM and IgD on naïve B cells to expression of one of the downstream isotypes: IgG, IgA, or IgE.

Throughout the differentiation process, the surface-bound immunoglobulin must perform two functions: signal transduction and the transport of captured antigen to an endosomal compartment for processing and presentation on the cell surface in association with major histocompatibility complex class II. But how are these dual requirements to be fulfilled in the face of the changing character of the immunoglobulin molecule, as the joint expression of IgM and IgD isotypes on naïve B cells changes to IgA, IgG, or IgE isotypes on the mature B cell?

The answer to this question at first appeared to be quite straightforward. In order to be expressed on the B cell surface, most immunoglobulins must be associated with two other polypeptides, Ig-α and Ig-β (see the figure) (*5, 6*). Both Ig-α and Ig-β contain a sequence (called ITAM) that can cause activation of protein tyrosine kinases, such as Syk and Lyn, accounting for the ability of isotype-switched B cells to signal in response to antigen (*7*). In addition, the cytoplasmic tails of Ig-α and Ig-β are sufficient for the internalization of surface proteins and their targeting to endosomal compartments for processing (*8*). It had been assumed, therefore, that both signaling and antigen-processing functions served by transmembrane immunoglobulins would be dependent on these unchanging accessory polypeptides. But the new results in this issue and elsewhere (*1–4*) show that the situation is far from being this simple and is therefore certain to be of even greater interest.

In these experiments, the cytoplasmic tails of the IgGs and IgE have been manipulated. Mouse and human IgM and IgD have a three–amino acid tail (Lys-Val-Lys), that of IgA has an additional 11 amino acids, and those of the IgGs and IgE are extended by 25 amino acids (*6*). The cytoplasmic tails of the IgG subclasses have many amino acids in common with each other and somewhat fewer in common with IgE.

The function of the conserved portion of the IgG2a cytoplasmic tail was investigated by Weiser *et al.* by using site-directed mu-

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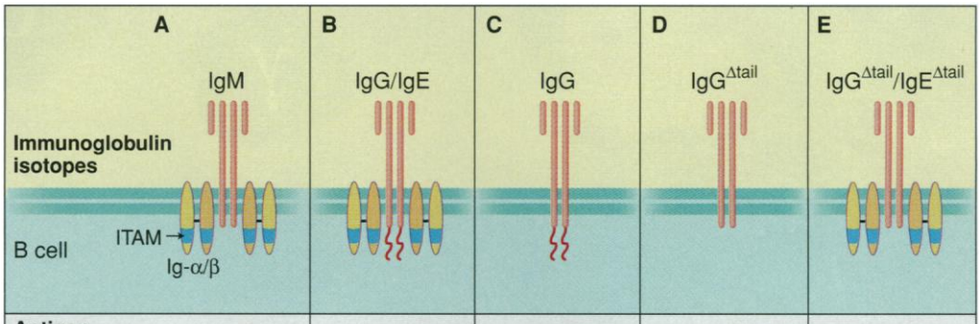
tagenesis to either delete or alter the sequence and then assessing the consequences in an in vitro antigen-presentation assay (*1*). The results were quite clear-cut: Without its cytoplasmic tail, IgG2a could be expressed on the cell surface in association with Ig-α/β, but could not present antigen to an appropriate helper T cell line (see the figure). By way of corollary, a chimeric molecule containing an antibody Fv segment, lacking a cytoplasmic tail, and expressed on the cell surface without Ig-α/β could mediate antigen presentation by the addition of the γ2a cytoplasmic tail. The ability to present antigen was also conferred on the chimeric protein by addition of the cytoplasmic tail of Ig-α and did not depend on the ITAM sequence required for signal transduction. Given this result, it is somewhat curious that antigen presentation of the tailless IgG2a was not rescued by its association with Ig-α/β.

Knight *et al.* analyzed the cytoplasmic tail of human IgG1 and found that this sequence mediated both internalization and targeting to an endosomal compartment for antigen processing and subsequent presentation (*4*). By comparing the reconstituted IgG1 in cell lines either expressing or lacking Ig-α/β, Knight *et al.* found no difference in the efficiency of antigen presentation as long as the cytoplasmic tail was intact (see the figure). A complete IgG1 presented antigen equally efficiently in the presence and absence of Ig-α/β. An IgG1 lacking a cytoplasmic tail, however, presented antigen 1/10th as efficiently in the absence of Ig-α/β, a drop that was not apparent when Ig-α/β was present.

The in vivo correlate of these experiments is reported by Kaisho *et al.* and Achatz *et al.*, who used gene targeting to generate mice homozygous for alterations to the cytoplasmic tails of IgG1 and IgE, respectively (*2*, *3*). Both groups created truncation mutations in which the cytoplasmic tails of the memory isotypes became identical to that of IgM, and both groups created mice in which both the cytoplasmic and transmembrane regions were deleted. In these two sets of mice, serum concentrations of IgG1 were reduced by a factor of 70 in the deletion mice and 24 in the truncation mice, whereas concentrations of IgE were reduced by a factor of 50 and 2 in the deletion and truncation mice, respectively. The nontargeted isotypes were present in normal amounts. Immunization of the IgG1-targeted mice with a T cell–dependent antigen resulted in 1/100th and 1/10th the amount of IgG1 in the deletion and truncation mice, respectively. The mice carrying the manipulated IgE genes also could not respond properly to Tcell–dependent antigens; this was evident in both the primary and secondary responses. Kaisho and his colleagues could detect surface IgG1-positive B cells in the truncation mice, but the frequency was 1/25th that in controls (*2*). The reduced frequency of isotype-switched B cells could be due to a defect in isotype switching itself rather than being a postswitching problem. This possibility was excluded by in vitro experiments showing that switching to the targeted isotypes occurred normally.



Immunoglobulin anatomy. (**A**) Upon binding of antigen, B cells expressing IgM efficiently process and present antigen and transduce a signal. Both of these functions are properties of the coreceptors Ig-α and Ig-β, the ITAM sequences of which are indicated. Upon isotype switching, the B cell receptor may either include Ig-α/β (**B**) or it may not (**C**). If Ig-α and Ig-β are present in the complex, then both signaling and presentation occur optimally. If the isotype is IgG and Ig-α/β are absent, then presentation can still occur but signal transduction cannot. (**D**) Antigen presentation in the absence of Ig-α/β by IgG is dependent on its cytoplasmic tail for full efficiency. (**E**) This deficiency can be restored in some cases (depending on the isotype) by association with Ig-α/β.

The phenotype of mice lacking the cytoplasmic tails of IgG1 and IgE is consistent with the isotype-switched B cells failing to receive adequate T cell help because of their inefficiency in antigen processing and presentation. That the deficiency of the IgE truncation mice should be less severe than that of the IgG1 mice may well reflect the ability of Ig-α/β to mediate antigen processing and presentation even in the absence of the cytoplasmic tail. Recall that Weiser *et al.* found that Ig-α/β could not rescue antigen presentation by a tailless IgG2a (*1*), whereas Knight *et al.* reported complete rescue for human IgG1 (*4*). The phenotype of the deletion mice is more severe because of the absence of surface immunoglobulin after isotype switching.

These observations raise interesting questions about the response of memory B cells to antigen and the targeting of immunoglobulin to the endosomal pathway. IgG1 expressed by memory B cells presumably exists in two forms, either in association with Ig-α/β or not. Upon exposure to antigen, those IgGs complexed with Ig-α/β will both transduce a signal and target antigen for processing and presentation, whereas those IgGs not so attached will generate no signal but will still result in antigen presentation. This suggests that by altering the ratio of Ig-α/β to IgG, a memory B cell could change the number of antigen receptors involved in signaling but not alter the cells' ability to present antigen for T cell help. It is not yet clear whether this might occur or what the function of this regulation might be.

Equally intriguing is the mechanism by which targeting of the immunoglobulin molecules to endosomal compartments occurs. The studies of Weiser *et al.* and Knight *et al.* implicate a tyrosine in the cytoplasmic tail as important for targeting to the endosomal pathway, possibly because of the secondary structure assumed by this region. It remains to be resolved whether the internalization and targeting apparently mediated by either Ig-α and Ig-β (*8*), or that mediated by the ITAM sequence of FcγRIII (*9*), occurs by the same mechanism as that mediated by the cytoplasmic tail of IgG. The new results illustrate a valuable approach to solving this problem by identifying a critical sequence motif.

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