

IMMUNOLOGY

Square-Dancing Antibodies

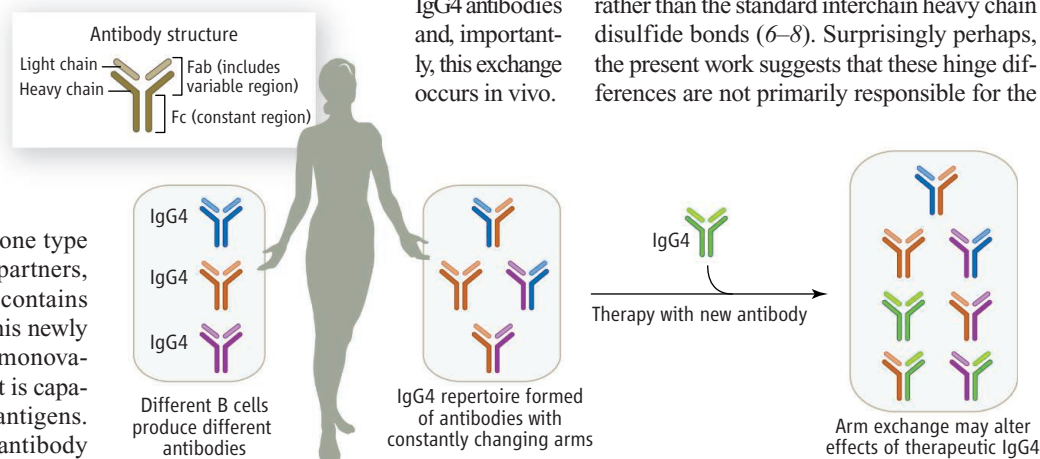
Dennis R. Burton and Ian A. Wilson

Antibodies are among nature's most versatile molecules. The classic Y-shaped molecules can recognize essentially any antigen by the variable tips of the Fab (fragment antigen binding) arms, whereas the Fc (fragment crystallizable) stem recognizes effector molecules that help eliminate antigen (see the figure). The two Fab arms are identical, allowing antibodies to bind bivalently to repeating antigens on, for example, microbial pathogens or tumor cells. This arrangement enhances the affinity of antibody for antigen and allows antibodies to cross-link antigen molecules under certain conditions. Identity of the Fab arms is expected because an antibody is a tetramer of two identical dimers. However, on page 1554 in this issue (1), van der Neut Kolfshoten *et al.* show that one type of antibody can exchange dimer partners, generating a hybrid antibody that contains two different Fab arms. Although this newly formed antibody can only bind monovalently, even to a repeating antigen, it is capable of cross-linking two different antigens. The consequences of such hybrid antibody formation are very interesting, but with some potentially serious ramifications.

The antibody molecule that exchanges Fab arms is human immunoglobulin G4 (IgG4). IgG, the predominant antibody in the serum, consists of four subclasses in humans. The least abundant subclass is IgG4, which is present in serum at a concentration of about 0.1 to 0.5 mg/ml (2). The IgG subclasses differ most in their Fc regions, and IgG4 notably has an Fc that interacts poorly with effector systems of the immune response, such as complement and Fc receptors expressed by certain white blood cells. This property has made IgG4 a favorite for therapeutic applications in which antibody is required to bind to a target, but not trigger effector activities.

Previously, it was noted that sera from donors making IgG4 antibodies to both house dust mite and grass pollen can cross-link these

two antigens, whereas sera from donors that make antibodies to one antigen alone cannot (3). Further, whereas most IgG molecules precipitate antigens when mixed in the right proportions, IgG4 antibodies do not (4). Some interesting hypotheses (5) were advanced to interpret these data, but this new study convincingly explains the behavior of IgG4. In particular, addition of a reducing agent, such as reduced glutathione, promotes exchange of Fab arms in vitro for both polyclonal and monoclonal IgG4 antibodies and, importantly, this exchange occurs in vivo.



Partners in arms. (Top) The Y-shaped structure of an IgG molecule is composed of two identical heavy (H) chains and two identical light (L) chains, resulting in two identical antigen-binding sites at the tips of the Y. However, an H-L unit of one IgG4 molecule can exchange with that of another IgG4 to produce a hybrid molecule with different antigen specificities. Because the Fc parts of two IgG4 molecules are identical, their Fab arms are effectively swapped. **(Bottom)** The exchange of Fab arms between IgG4 molecules is dynamic. An initially homogeneous IgG4 antibody, when administered to a human, will begin swapping arms. Partners exchange constantly, as this "dance" progresses.

An insightful application of this Fab arm exchange is illustrated in a monkey model of the human autoimmune disease myasthenia gravis. The disease presents as muscle weakness associated with autoantibodies to acetylcholine receptors (AChR) expressed on muscle cells. van der Neut Kolfshoten *et al.* show that a human IgG1 antibody to AChR isolated from a patient with myasthenia gravis induces disease symptoms in monkeys presumably by cross-linking AChR molecules. However, a human IgG4 antibody with identical binding properties to AChR as the IgG1 antibody does not cause disease. Furthermore, the IgG4 antibody protects against IgG1-mediated disease likely by displacing IgG1 and binding monovalently to AChR.

Antibody therapies need to take account of a subclass of immunoglobulin G that can swap subunits in vivo.

How then does this exchange of Fab arms occur between IgG molecules? Dimerization of IgG heavy chains is thought to be driven by the pairing of the third constant domain (C_{H3}) of each heavy chain. This pairing is usually stabilized further by interheavy chain disulfide bridges some distance away in the hinge region. In IgG4, the hinge region has the amino acid sequence Cys-Pro-Ser-Cys, compared to Cys-Pro-Pro-Cys in IgG1, and this difference has been linked to a tendency of IgG4 to form novel intrachain disulfides in the hinge region rather than the standard interchain heavy chain disulfide bonds (6–8). Surprisingly perhaps, the present work suggests that these hinge differences are not primarily responsible for the

exchange of Fab arms but, instead, implicates the C_{H3} domains.

Sequence differences in the C_{H3} domain of IgG4 and IgG1 are limited to four residues; most notably, an arginine at position 409 at the interface between C_{H3} domains in IgG4 is replaced by a lysine in IgG1, and this may be crucial for Fab arm exchange. Although this appears to be a rather conservative mutation, arginine-to-lysine substitutions can have large effects on protein-protein interactions in other systems (9, 10). Clearly, further mutagenesis studies are warranted to address this possibility.

What are the ramifications of Fab arm exchange for antibody-mediated immunity? IgG4 is produced particularly in response to high doses of protein antigen, and there has been controversy over the abil-

ity of IgG4 to dampen IgE responses when the antigen is an allergen and also whether this dampening effect has clinical utility (5, 11). Any effects are likely due to competition between IgE and IgG4 to bind antigen, but this should now be considered in the light of the findings of van der Neut Kolfshoten *et al.*

Several IgG4 molecules are in clinical use or in clinical trials. In some instances, the hinge region of IgG4 has already been “stabilized” by mutation to the IgG1 Cys-Pro-Pro-Cys sequence, and it is probable that Fab arm exchange has been reduced or even abrogated.

In other instances, a wild-type IgG4 molecule may have been used, and the possibility that Fab arm exchange could contribute to adverse effects in IgG4 therapy (12, 13) should be explored immediately.

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GEOCHEMISTRY

New Players in an Ancient Cycle

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Microorganisms such as cyanobacteria, sulfate reducers, and methane-producing archaea can be traced back to ~2.7 billion years ago on the basis of chemical biomarkers, microfossils, and stable isotopes. Farther back in time, the evidence for microbial metabolism resembles a puzzle in which most pieces are missing. On page 1534 of this issue, Philippot *et al.* (1) present a new piece: isotopic evidence for sulfur disproportionation, a little-explored metabolic process, in a 3.5-billion-year-old marine deposit.

The geological record of stable sulfur isotopes holds vital clues about Earth's history. Sulfate-respiring bacteria and archaea in marine sediments favor ^{32}S over ^{34}S in their

reduction of sulfate, yielding ^{34}S -depleted hydrogen sulfide; this signal can be traced back 2.5 to 2.7 billion years. Sulfate reducers only discriminate the isotopes at sulfate concentrations above ~1 millimolar. The isotope signal thus documents a sulfate-rich ocean (2).

The absence of this isotopic signal before 2.7 billion years ago (3) could mean that sulfate levels were low or that sulfate reduction had not yet evolved. In support of the former explanation, Shen *et al.* (4) have reported evidence that sulfate reducers existed 3.5 billion years ago. The authors found ^{34}S -depleted sulfide associated with a barium sulfate bed from North Pole, Western Australia.

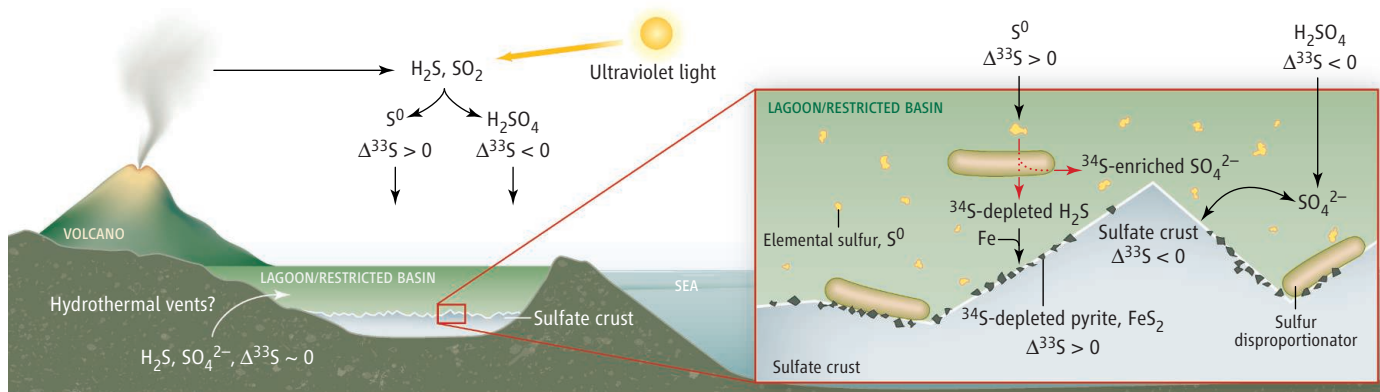
The results of Shen *et al.* (4) are one of the two oldest reported datings of a specific energy metabolism. More recently, methanogenesis was dated to the same age (5). Carbon isotope analysis has provided evidence for

Isotope data from Australia provide evidence for the existence of bacteria that used elemental sulfur in metabolic processes about 3.5 billion years ago.

biological carbon fixation by 3.8 billion years ago (6), but carbon fixation may be linked to different types of energy metabolism. Philippot *et al.* have now repeated the sulfur isotope analyses from North Pole with new samples, finding slightly more ^{34}S -depleted sulfide than the previous study. They also extended the analysis to ^{33}S , which gives information about the sulfur source. The results show that the simplest explanation is not always the right one.

For elements with several stable isotopes such as sulfur, isotopic fractionation in aqueous processes—including enzymatic ones—depends closely on the mass differences between the isotopes; for example, the fractionation between ^{33}S and ^{32}S is 0.515 times that between ^{34}S and ^{32}S (7). In contrast to this mass-dependent fractionation, atmospheric photochemical

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The sulfur cycle at North Pole according to (1). Atmospheric reactions of sulfur gasses induce a mass-independent fractionation with positive ^{33}S anomalies ($\Delta^{33}\text{S}$) in elemental sulfur (S^0) and negative anomalies in sulfuric acid (H_2SO_4). Sulfur and sulfate are deposited in a shallow, sulfate-rich basin.

(Inset) Elemental sulfur is disproportionated by microbes, with mass-dependent fractionation into ^{34}S -depleted hydrogen sulfide and ^{34}S -enriched sulfate. Hydrogen sulfide precipitates as ^{34}S -depleted pyrite on the surface of the crust, whereas the sulfate is diluted into the large sulfate pool.

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