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EDITORIAL REVIEW

The molecular basis for a polyspecific antibody

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Following the seminal work of Landsteiner and colleagues [1], who demonstrated that specific antiserum could be raised to different haptens, it was generally agreed that antibodies were specific for the immunizing antigen. This view has subsequently been strengthened by the advent of highly specific mouse monoclonal antibodies. Contrary to this point of view, were reports of myeloma proteins, the MoAb produced by human B cell lymphomas, which were able to recognize multiple antigens [2]. Such 'polyreactive' antibodies have also been subsequently described in healthy individuals or unimmunized animals [3,4]. Limiting dilution analysis following polyclonal activation and transformation of human peripheral blood B lymphocytes with Epstein–Barr virus (EBV), revealed that between 3% and 13% of the IgM-secreting clones produced antibodies reactive against multiple autoantigens including ssDNA, IgG Fc, thyroglobulin or insulin [5]. These antibodies also recognize protein and carbohydrate antigens on bacteria and viruses. Whilst many of these antibodies are of the IgM isotype, IgG and IgA polyreactive antibodies can also be readily detected in the serum of normal healthy animals and man.

The affinities of polyreactive antibodies tend to be low, with a dissociation constant (Kd) of between 1×10^{-3} and 1×10^{-7} M, though some have a Kd within the range expected for monospecific antibodies, i.e. 1×10^{-7}–1×10^{-11} M. However, since many of these polyspecific antibodies are IgM, existing as pentamers, the actual avidity may be significantly higher. Sequence analysis of the variable (V) regions of polyreactive antibodies has revealed that they are often in germ-line configuration, without N-nucleotide insertions at the V_H–DJ_H junctional regions, and have limited or absent point mutations at the complementarity determining regions (CDR). This suggests that these antibodies have not undergone the antigen-driven somatic mutations which are associated with achieving high-affinity antibodies. Analysis of the B lymphocytes producing these antibodies identified CD5, $CD45RA^{lo}$ as a marker for these cells, which constitute up to 25% of the circulating B lymphocyte population [6]. Additionally, $CD5^{-}CD45RA^{lo}$ B lymphocytes have also been shown to produce polyspecific antibodies, and these two populations have been described as B1 lymphocytes, to distinguish them from the B2 lymphocytes which are $CD5^{-}$ $CD45RA^{hi}$ and tend to secrete high-affinity monospecific antibodies following contact with antigen.

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DO POLYREACTIVE ANTIBODIES HAVE A BIOLOGICAL ROLE?

It has been suggested that by forming immune complexes with autoantigens released following tissue damage or re-modelling, these antibodies may be involved in the clearance of cellular antigens. Furthermore, since they often recognize viral and bacterial antigens, polyreactive antibodies may represent a first line against infection in the absence of previous exposure to antigen. A role for natural antibodies in the prevention of tumour growth has also been suggested [7], which is supported by the low tumour resistance of B lymphocyte deficient *xid* mice [8]. The presence of polyspecific antibodies which recognize a whole range of cellular and soluble autoantigens in the serum of healthy individuals and unimmunized animals, suggests that they do not necessarily result in autoimmune disease. However autoantigen-driven somatic mutations, presumably with the help of (autoreactive) T lymphocytes, may result in an antibody with sufficient affinity to contribute to autoimmune disease [9].

HOW THEN CAN POLYSPECIFICITY BE ACHIEVED?

One possibility is that these antibodies merely recognize cross-reactive epitopes in different antigens, though this would seem unlikely for most polyspecific antibodies, given the disparate nature of the multiple antigens recognized. The inability to demonstrate cross-reactive epitopes to account for polyspecificity raised the question of whether it was the physio-chemical properties of these antibodies which were responsible for polyspecificity. However, using two-dimensional electrophoresis to measure the charge of the heavy and light chains of monoreactive and polyreactive antibodies failed to demonstrate an obvious difference, although as pointed out by the authors, such a technique would fail to detect local charge differences [10]. The same group examined the specificity of the Fab'_2 fragments of these natural antibodies, which demonstrated that polyspecificity was retained, implying that either local charge differences within the Fab fragment, or even the paratope itself, may be responsible for this phenomenon. Indeed, a rabbit antiserum that recognized the idiotope on a mouse IgM polyspecific antibody, also recognized a high incidence of other mouse, rat and human polyspecific antibodies [11]. A recent comparison of the nucleotide sequence of a human poly- and monoreactive IgM antibody failed to detect any coding differences in the constant regions of these anti-

bodies, again confirming that the V regions are important in conferring polyspecificity [12].

The antigen-binding pocket of immunoglobins is lined by the three hypervariable loops, or CDRs, of the variably heavy (V_H) and light (V_L) domains [13]. That the specificity of a monospecific antibody is determined largely by these CDRs has been elegantly shown by the ability to transfer antibody specificity by grafting these regions into an unrelated antibody [14]. Does the same hold true of a polyspecific antibody? Bohn *et al.* have conducted a series of experiments which are pertinent to this question, the results of which are published in this issue [15]. The group have for some time been characterizing a human x mouse heterohybridoma made from the splenic lymphocytes of a patient suffering from chronic idiopathic thrombocytopenia. The purified IgM antibody from this hybridoma (CB03) binds to a cell surface tumour-associated antigen, resulting in growth inhibition and an increase in MHC antigen expression *in vitro*. The antibody additionally recognizes tetanus toxoid, ssDNA and dsDNA, thus fulfilling the characteristics of a polyspecific MoAb. A single chain fragment (scFv) of CB03, V_H-linker-V_L, was expressed in *Escherichia coli*, and using surface plasmon resonance to measure the binding constants of the scFv for different antigens, it was demonstrated that polyspecificity was a property of the variable domains [16]. Additional scFv constructs have subsequently been made to determine the molecular basis for the polyspecificity of this antibody. These involved changing the two somatic mutations in the CDR1 and CDR2 V_H domain of CB03 to the germ-line sequence, or swapping the CDR3 region of CB03 with the CDR3 from another polyspecific IgM antibody (CB253), which utilized a closely related V_H gene, but did not bind to tumours. The V_H constructs were linked to the V_L domain of CB03 antibody. The scFv of CB03 retained the characteristics of the parental antibody in terms of its polyspecificity and tumour binding. However, replacing the point mutations in the CDR1 and 2 with the germ-line sequence resulted in a loss of tumour binding but retained polyspecificity, suggesting that tumour reactivity was the result of somatic mutations. If the CDR3 on the scFv of CB03 was changed to that of CB253, tumour binding was retained, consistent with the importance of CR1 and CR2 in this regard, but the ability to bind to dsDNA was lost, binding to ssDNA reduced and tetanus toxoid unaffected. Thus it would appear that changing the CDR3 critically affects some of the polyspecific properties of this antibody.

The CR3 of CB03 is unusual in that it is both extended in length [17], and rich in arginine and asparagine residues, properties often seen in the V region of other polyspecific antibodies [18]. It is known that these amino acids are important in DNA–protein interactions [19,20], and that inserting arginine residues into the V_H may increase the affinity of anti-dsDNA antibodies [21]. It has also been suggested that the extended CDR3 would create a larger antigen-binding pocket, providing the structural basis for polyreactivity [17]. Taken together, these observations may explain the dependency of the CDR3 of CB03 for dsDNA reactivity. The finding that tumour reactivity is dependent on somatic mutations within the CDR1 and 2 may be interpreted as evidence that this polyreactive antibody has undergone some degree of antigen-driven selection. This conclusion is consistent with the presence of somatic mutations in some human IgG polyspecific antibodies which have additionally undergone class switching [22]. Thus polyspecific antibodies may, in part, represent 'immature' antibodies which have not undergone antigen-driven maturation, due to the absence either of antigen or of appropriate T lymphocyte help, and that their polyspecificity results from an antigen-binding pocket which has not been fine-tuned to a particular antigen.

Since their first description, polyspecific antibodies have been viewed with considerable scepticism, and the biological significance of these typically low-affinity antibodies questioned by mainstream immunologists. Whilst the findings of Bohn *et al.* do not address these concerns, they have at least provided the molecular basis for polyspecificity for one such antibody. Should it be subsequently proven that other polyspecific antibodies also bind multiple antigens through their paratope, then it is the question of a biological function for these antibodies that remains to be resolved.

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