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When Antibodies Are Antigens*

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In the winter of 1945/46, I initiated my collaboration with Ludwik Hirszfeld in Wrocław, Poland. At that time Hirszfeld was 61 years old. He was famous for his discoveries on the heredity and anthropology of human blood groups, the description of a paratyphoid C bacillus, studies on the serological structure of pathological tissues, as well as pioneering concepts on constitutional serology. He was a leading personality in Polish medicine. I was 26 and had just obtained my medical diploma. I was very happy when Hirszfeld appointed me assistant professor in his Department of Medical Microbiology at the University of Wrocław. In the spring of 1946, I started teaching medical students in the laboratory and I also conducted the examination of students whom we had accepted to the school after several years of interruption of their studies because of the war. As could have been expected, many of them were poorly prepared. One student, asked by me what he knows about antibodies, answered without too much hesitation: "Antibodies are antigens". It goes without saying that I was appalled with his ignorance; this was the worst answer I had ever heard. I certainly did not anticipate at that moment that for all my research life I would be following the student's statement by studying those situation in which "antibodies are antigens".

Anti-Antibodies

The term anti-antibody has been used in a broad sense, referring to all possible antibodies reacting with other antibodies, the latter acting as antigens.

In this sense, antibodies against immunoglobulins of unknown antibody specificity are also anti-antibodies. As a result of my reviewing the classical immunological literature on this subject, I became interested in two avenues of approach. The first followed the classic hypothesis of BORDET[2] that an antibody undergoes molecular transformation in the course of the reaction with its antigen. Since this hypothesis had not been confirmed experimentally up to the 1950s, it was largely abandoned. However, I considered it possible that serological procedures might detect the appearance or exposure of novel antigenic site on the antibody molecule that had been modified by it reaction with the antigen. The second approach was stimulated by the hypothesis presented by EHRLICH and MORGENROTH[4], which postulated anti-antibody formation against the antibody-combining site. These authors theorized that such "true" anti-antibodies may play important homeostatic roles in preventing the formation of harmful autoantibodies. Up to that time such "true" anti-antibodies had never been detected and in the 1946 LANDSTEINER[12] wrote: "there is no evidence for the existence of true anti-antibodies which would bear a relation to the specific antibody function". Being young and bold, I wanted to engage in this line of investigation, which appeared difficult but fascinating.

In studies performed in Hirszfeld's department with Luszczynski and Dubiski, I embarked on the production of anti-antibodies by immunization with immune complexes[18]. Rabbit erythrocytes were agglutinated by pooled normal human serum, after we had found that such serum had high titers of natural anti-rabbit hemagglutinins. Washed agglutinates were injected intravenously into rabbits, surprisingly without doing any obvious harm to these animals. The immunized rabbits formed very strong antibodies to human antibodies. In spite of extensive studies, however, we could not obtain any evidence that these anti-antibodies distinguished the molecular transformation of human antibodies in their reaction with rabbit erythrocytes, or otherwise,

* Dedicated to the memory of my beloved teacher Ludwik Hirszfeld.

that they had properties of true anti-antibodies combining with the antibody site specific for rabbit erythrocytes. We believed that the lack of success in these studies might have been due to "faulty perspective", in that rabbit is a species too remote form man to distinguish subtle differences in the structure of human immunoglobulin. As a consolation prize, we found that we had produced excellent anti-globulin reagents that could be used in our own and other blood group laboratories. Several years later, it was found our anti-sera contained predominantly antibodies to human IgG, but also antibodies to human IgM and complement.

Next, in collaboration with Dubiski and Woźniczko, I conducted, simple and inexpensive experiments on human material following the assumption that antigen--antibody complexes formed *in vivo* stimulate formation of anti-antibodies that react with an antibody combined with its antigen but not with an antibody free in the serum[16, 17]. We theorized that such anti-antibodies would be found in some proportion of the human population, primarily in those individuals who had experienced some persistent infections. As an immune complex for *in vitro* test, we used human Rh-positive erythrocytes sensitized by incomplete Rh antibodies, expecting that human sera with anti-antibodies would agglutinate such erythrocytes. We found that, indeed, about 5% of sera tested produced agglutination even though only about 1 in 10 of positive sera had agglutinating titer exceeding 100. The properties of the tested anti-antibodies were precisely such as we had predicted in that they reacted with Rh antibodies and other human antibodies which were altered by the impact of serological reaction but not with "free" antibodies in the serum. We could demonstrate this in a persuasive way by "mixed serum reaction", in which a mixture of a serum containing incomplete Rh antibodies with an anti-antibody-containing serum acted as a "complete" Rh serum in that it produced agglutination of Rh-positive erythrocytes. Obviously, our anti-antibody was not neutralized by "unaltered" Rh antibodies or by normal gamma globulins of Rh antiserum. It was quite obvious that in the "mixed serum reaction", Rh antibodies combined with the erythrocytes, which resulted in the appearance of novel antigenic sites and, then, anti-antibodies bound to these novel sites produced agglutination.

It was subsequently shown that anti-antibodies detected by us are of IgM isotype and that they react with IgG antibodies. This study rather clearly showed that, as predicted by Bordet, an antibody molecule undergoes transformation in serological reaction, a finding which was subsequently substantiated by physiochemical studies of Ishizaka and Campbell[8].

Rheumatoid Factor and Milgrom Factor

In reviewing the bibliography, we came across publication on antiglobulin factors in sera of patients with rheumatoid arthritis[24, 26]. It was rather obvious to us that this "rheumatoid factor" was different from our anti--antibody, since the former had been detected in reaction with erythrocytes sensitized by rabbit antibodies, whereas the latter reacted with erythrocytes sensitized by human but not by animal antibodies. Sebsequently, Franklin et al.[5] showed that rheumatoid factor was a 19S serum protein, and Waller and Vaughan[27] as weel as Grubb[6] found the rheumatoid factor would also agglutinate Rh-positive erythrocytes sensitized by incomplete Rh antibody in a similar way to our anti-antibody. Significantly, however, the reactions of rheumatoid factor in this test could be neutralized quite readily by many whole human sera or fraction II of human serum, which was not the case with respect to our anti-antibody. To distinguish it from the rheumatoid factor, Kunkel and Tan[11] called the anti-antibody described by us the "Milgrom factor".

Experimental Formation of "True" Anti-Antibodies

In studies performed in collaboration with Dubiski, we wanted to assess whether immunization of a rabbit with a powerful antigen conducted over a prolonged period of time would result in the formation of anti--antibodies[15]. We selected for this immunization guinea pig leukocytes only because we were quite poor at that time and we needed antiserum to this antigen for other studies. After immunization, which lasted for 4 months, we concluded: "the first antibody to appear was anti--leukocyte antibody. Further injection of leukocytes caused the *in vivo* reaction between these cells and their corresponding antibody. In this reaction the antibody was presumably altered and stimulated the production of "anti-antibody". To the best of my knowledge this was the first demonstration that prolonged immunization with an antigen is accompanied by formation of anti-antibodies.

In the same paper[15], we also reported the results of immunization with antigen-antibody complexes. In contrast to out previous experiments, in which rabbits were immunized with complexes formed by human antibodies, in the present study the antibodies were of rabbit origin. We produced at first rabbit anti-sera to *Proteus* as well as to *Escherichia* bacilli, and then we injected rabbits with bacilli agglutinated by these anti-sera. The anti-antibodies obtained by this immunization appeared to have characteristics of "true" antibodies. We wrote: "...anti-anti-

body against *Proteus* antibody and that against *Escherichia* antibody are not cross-absorbed. Perhaps this indicates differences in antigenic structure of rabbit antibodies of different specificity".

A few month after publication of this paper, I left Poland. Working at the Pasteur Institute in Paris in the fall of 1957, I had the opportunity to discuss my studies on the antigenicity of antibodies with Jacques Oudin. Then, after my arrival in the USA in the spring of 1958, I discussed these studies with Henry Kundel of the Rockeffeller Institute. Both Oudin and Kunkel were interested in my studies, especially in the observation on "true anti-antibody". A few years later they published their studies on such antibodies, which they named "idiotypic antibodies"[10, 22]. These studies stimulated JERNE[9] to formulate the theory of idiotype networks to advance the concept of "internal images". With the accumulation of experimental material, however, evidence was presented that idiotypic specificity is closely related to but not completely identical with the structure of the antibody combining site. I myself have been preoccupied in subsequent years with studies on anti-antibodies combining with altered immunoglobulins and did not return to studies on "true" anti-antibodies.

Studies on Rheumatoid Factor

In the fall of 1957, I participated in the 6th Congress of the European Society of Hematology held in Copenhagen. This was the first international meeting that I had ever attended, since I was not permitted to travel abroad when I was working in communist Poland. During the meeting in Copenhagen, I had the opportunity to participate in discussions on rheumatoid factor. Strangely enough, the investigators conducting experiments on this factor were very reluctant (to put it mildly) to consider that this factor is an antibody, and I defended single-handedly its antibody nature.

Upon my arrival to the USA in 1958, I joined Ernest Witebsky's Department of Bacteriology and Immunology in Buffalo, New York. This department was occupied with studies on autoimmunity and autoimmune diseases in wake of the discovery by ROSE and WITEBSKY[25] of experimental autoimmune thyroiditis, and Witebsky was very interested in my studies on rheumatoid factor. I told him that I assume that rheumatoid factor was an antibody produced in a similar way as I had proposed for my anti-antibody, i.e. as a result or prolonged exposure of the patients to antigen-antibody complexes, a stereotype of reaction rather than an immune response specific for rheumatoid arthritis. To challenge this hypothesis, Witebsky suggested that I should examine sera of patients with subacute bacterial endocarditis. I tested 7 such sera for rheumatoid factor and found that all were positive[29]. In the meantime, also, our study on prolonged immunization of rabbits with a strong antigen was followed by several other investigators and they showed that such immunization is indeed accompanied by the formation of anti-antibodies[1].

Still, we needed direct assurance that autologous IgG becomes antigenic as a result of molecular transformation. We decided to perform these experiments in rabbits by injecting each animal with its own denatured gamma globulin[20]. To this end, serum samples obtained from several bleedings of individual rabbits were preserved at –20ºC, pooled and precipitated by ammonium sulfate. The gamma globulin preparations, denatured by the isolation procedure as well as by freezing and thawing, were injected back into the serum donor in the several inoculations. This immunization resulted in the formation of antibodies with serological features resembling the human rheumatoid factor. Interestingly, these anti-antibodies produced much stronger reactions with the gamma globulin of foreign species origin than with that of rabbit origin. Apperently, the alteration of IgG created or exposed epitopes which are normally present in IgG of other species. These results were confirmed and extended by MCCLUSKEY et al.[13]

Is Rheumatoid Factor a Multispecific Antibody?

As mentioned before, rheumatoid factor was studied primarily in reactions with IgG of human and rabbit origin. Studies performed by several investigation in the 1950s seemed to indicate that rheumatoid factor is primarily directed against human IgG and that its reaction with rabbit IgG should be considered a cross-reaction. Our studies[21], performed by means of mixed agglutination showed, however, the most rheumatoid sera contain three clearly distinguishable populations of rheumatoid factors: a) those reacting with human IgG only, b) those reacting with human and rabbit IgG and c) those reacting with rabbit IgG only. These studies were confirmed and extended by other investigators[28]. On the basis of my previous studies[14], we expressed our hypothesis that factor (b), reacting with both human and rabbit IgG, is a multispecific antibody having separate or overlapping combining sites for each of these two IgG. This thesis attained credence in connection with studies conducted by MILGROM and SWIERCZYNSKA[19] on monoclonal rheumatoid factor. This study is of particular interest on connection with the observations of HANNESTAD and JOHANNESSEN[7] which identified rheumatoid factors that combined not only with IgG, but also cross-

-reacted with nuclear antigens. It appears quite likely that Hannestad factors are multispecific antibodies.

Pathogenicity of Anti-Antibodies

In spite of many attempts, the occurrence of rheumatoid factor could not be related to pathological lesions in the joints of patients with rheumatoid arthritis. Also, our animal studies failed to find any lesions in the joints of rabbits that produced anti-globulin antibodies of various types. On the other hand, in our studies in the 1980s we noticed that rabbits exposed to immunization with denatured or cationized rabbit IgG developed glomerular lesions with linear and granular deposits of rabbit IgG on glomerular capillary walls[3]. In some of these rabbits proliferative glomerulonephritis was noted. This observation was consistent with our study on human glomerulonephritides, which brought evidence that immune deposits on glomerular basement membranes are formed by human IgG and that they may be dissolved in antigenic excess provided dirung the incubation of kidney sections in solution of aggregated human IgG[23]. These studies as well as observations by other investigators seem to indicate that rheumatoid factor play a significant role in the formation of pathogenic immune complexes. Interestingly, available evidence would indicate that the nephritogenic rheumatoid factor is of IgG rather than of IgM nature.

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