Immunoglobulin profiles of the chronic antibody response: discussion in relation to brucellosis infections

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Summary
The use of a sensitive and quantitative radioimmunoassay has enabled much finer dissection of the immunoglobulin-antibody profiles for an individual immune response. The kinetics of the response are considered in relation to the switch from IgM to IgG antibody production. In the domestic fowl, the kinetics of this switch varied with different antigens: whereas the response to a thymus-dependent antigen proceeded through a brief 19S response to a declining 7S response, the response to a thymus-independent antigen failed to switch from 19S to 7S for several weeks and consisted of repeated excursions of 19S antibodies. When injected intravenously and simultaneously, Salmonella adelaidae O (killed) organisms (thymus-independent) and sheep red cells (thymus-dependent) interact so that the response to the latter fails to switch from 19S to 7S and consists of repeated excursions of 19S antibody. The changed character of the sheep red cell response is interpreted as being due to lack of 7S antibody. Passive antibody to either sheep red cells or to S. adelaidae produced an inhibition of the sheep red cell response so that only one excursion of 19S antibody was observed.

Moreover, it has been postulated (Anderson et al., 1964; Reddin et al., 1965; Coghlan and Weir, 1967) that a determination of the immunoglobulin class of Brucella antibody present in serum allows determination of the stage of the disease, whether acute, subacute or chronic. Up to the present, the estimation of antibody to B. abortus in terms of immunoglobulin class has been deduced for clinical diagnostic purposes from the results of direct and indirect agglutination tests, in presence and absence of 2-mercaptoethanol and by the complement fixation test (Kerr et al., 1966a; b; 1968). In the present approach (Parratt et al., 1977) antibody in each of the three classes IgM, IgG and IgA has been determined directly by the method of radio-immunoassay; but before the results obtained in Brucella infections in man are discussed, consideration must be given to the control processes which determine the sequential production of the different immunoglobulin classes of antibody.

The response of man to all antigenic stimuli may involve the subsequent production of antibody in any or all of the immunoglobulin forms IgM, IgG, IgA, IgD and IgE. At present it is not understood why this antibody response needs to be so complex, although it is known that all these molecular forms of antibody have different heavy chains which confer different biological activities. It seems clear that evolutionary progress among the higher vertebrates has coincided with the progressive development of more immunoglobulin classes or subclasses of antibody. The first immunoglobulin class to be made in any immune response is IgM and the biosynthesis of other immunoglobulins is directly dependent on this prior IgM response. The clearest evidence of this is that antibody specific for the µ chain of IgM, injected into neonatal mice (Lawton et al., 1972; Manning and Jutila, 1972a, b; Murgita, Mattioli and Tomasi, 1973), or into chick embryos in the egg (Kincade et al., 1970; Kincade and Cooper, 1971) or used in lymphoid cultures in vitro (Pierce, Solliday and Asofsky, 1972) causes suppression of subsequent IgM production and also of other
immunoglobulins. The injection of antibody against the γ chain of chicken 7S Ig (IgY or IgG) will prevent subsequent production of IgG and IgA but allow production of IgM and the later switch from IgM to IgG occurs within the same plasma cell derivatives (plasmacytes). Although the data, from chickens, of Kincade and Cooper (1971) support the hypothesis that IgA production depends necessarily on a similar later IgG→IgA switch, this was not borne out by the data obtained by Martin and Leslie (1974) in chickens and by the in vitro data of Pierce et al. (1972). All of these experiments supported the possibility of a direct IgM→IgA switch. The most likely explanation for the effects of anti-γ antibody on the immune system is a reaction with IgM receptors on the surface of virgin precursor B cells, including those which would eventually give rise to IgA and IgG producers. Consideration of the immunoglobulin composition of sera must take account of immunoglobulin turnover. In man, IgG has the longest half-life (23 days approximately at a normal serum level of IgG) as compared with 6 days for IgA and 5 days for IgM. IgG will, therefore, tend to build up and persist, relative to other immunoglobulins, in the sera from chronic infections.

What controls the switch from one immunoglobulin class to the next? In the adult antibody response it has long been established that specific antibody can interfere with the production of the same specificity of antibody (Uhr and Möller, 1968). This demonstration usually involves the injection of an antiserum ( principally this is IgG antibody from an hyperimmune serum) before or at the same time as the injection of antigen. This suppresses both IgM and IgG responses, and has been shown to do this in a variety of animal species. More recently, the same suppressive action has been shown for IgA and IgE antibodies (Strannegard and Belin, 1970; Ishizaka and Okudaira, 1972).

The suggestion is that not only are the antibody responses IgM, IgG, IgA and IgE linked in succession but that negative feedback loops exist so that antibody of one class can suppress its own synthesis; the antibody of succeeding classes, especially IgG, can exert feedback control on antibody of the previous immunoglobulin forms. Clearly there are very many possibilities, but in order to make this presentation tolerable, let us limit consideration to IgM and IgG, with lesser attention to IgA.

Many sources state that both IgM and IgG antibodies have a suppressive action. In the mouse response to sheep erythrocytes (a thymus-dependent antigen at least at low antigen dosage), purified 19S antibody consistently increased the primary response. Antibody of the same specificity, which was 7S, consistently suppressed the response (Henry and Jerne, 1967); and these authors attributed the suppressive effects of 19S, described as others, due to varying degrees of contamination of the 19S preparations with 7S antibody. Others have found that while a really high dose of 19S antibody is suppressive, lower doses are able to enhance the antibody response (Möller and Wigzell, 1965). The present author’s own experiments in the domestic fowl, have consistently confirmed the ability of IgM antibody to produce positive rather than negative feedback. Figure 1 shows the results of increasing doses of IgM antibody given at the same time as the antigenic stimulus: all doses produced an increase in plaque numbers at 6 days of a primary response. It was also found that IgM antibody generally enhanced the response when injected over a range of different times relative to the antigen injection (Fig. 2). Contrariwise, the 7S antibody was always suppressive of the IgM plaque-response, being strongly so when given early in the response.

![Graph showing effect of increasing amounts of 7S, IgG or 19S, IgM antibody on the antibody response at 6 days as judged by the counts of plaque-forming units (PFU) in 10^4 spleen cells. Note that all doses of IgM antibody enhanced the response, whereas low doses of IgG antibody enhanced but higher doses produced increasing depression of the response. (Data from experiments with K. H. Nielsen.)](http://pmj.bmj.com/)

The ability of 7S antibody to produce negative feedback is governed by the avidity of the antibody produced. During the progress of an immunological response there is a progressive rise of antibody avidity, at least if the antigen dose is maintained low. High avidity antibodies are more competent to suppress the immunological response than low avidity antibody (Walker and Siskind, 1968). It is
easy with 7S IgG antibody to suppress the 19S IgM primary response; relatively easy to suppress with hyperimmune serum the 7S IgG primary response; but difficult to suppress the 7S IgG secondary response.

So far, the results quoted relate to thymus-dependent antigens. Thymus-independent antigens are generally regarded as stimulating B cells directly (without aid from macrophages or T-cells) and leading to responses which, at least initially, are exclusively 19S antibody. T-independent immunogens give us the opportunity to study antibody homoeostasis in the absence of 7S antibody. Figure 3 shows the response of individual chickens to an injection of $10^6$ heat-killed *Salmonella adelaeida*. The antibody which localizes to the surface of these bacilli (mainly anti-O) was estimated by a radioimmunoassay in which IgM or IgG antibody was measured independently of 7S antibody. It can be seen that the resulting IgM response is a cyclical sequence of several peaks of decreasing magnitude. They repeat every 10 days for four cycles before they become dys-synchronous in different animals. During this period of 19S antibody peaks, 7S antibody is undetectable but increases slowly from about 40 days.

The explanation of this antibody profile appears to depend on the following. The antigen generates a 19S antibody response which eventually reaches sufficient concentration to exert negative feedback. However, since there is no shift to 7S antibody and 19S antibody has a very short duration (half-life of 18 hr), suppression is temporary; also, since bacterial lipopolysaccharide is persistent within the tissues, another cycle of 19S antibody is irritated, and so on until 7S antibody makes its appearance in sufficient amounts to secure permanent negative feedback of the response. As would be expected, the cycles of 19S antibody can be brought to a

FIG. 2. Effect of a single dose of antibody (7S, IgG or 19S, IgM) given intravenously to a young fowl at various times from 1 day before to 6 days after an intravenous injection of 2 mg human serum albumin (antigen). The ordinate expressed the count of plaque-forming units from 10$^6$ spleen cells at 6 days after antigen. Note that 19S, IgM antibody always enhanced and 7S, IgG antibody always depressed the immune response. (Data from experiments with K. H. Nielsen.)

FIG. 3. Antibody responses of individual adult fowl to $10^6$ *Salmonella adelaeida* O micro-organisms injected i.v. 19S antibody levels were estimated by radio-assay in the five birds at times up to 65 days. Note the cyclical response of 19S antibody with peaks at days 4–7, 14–16, 25 and 35–37 days. (Data from White and Nielsen, 1975.)
halt by passive administration of 7S-antibody. An injection of 1 ml of 7S anti-O S. adelaide (Fig. 4a and b) promptly eliminates the cyclical activity of 19S antibody and secures negative feedback of the response.

The author believes that this model of a thymus-independent antigen is highly appropriate for most bacterial infections. Although the reason why has not yet been discovered, most of the significant molecules at the surface of bacteria appear to be thymus-independent antigens (lipopolysaccharide, carbohydrates of pneumococcal capsules, capsules of Klebsiella spp., polymerized flagellin, etc.). Moreover, in one system which used the T-independent S. adelaide together with T-dependent sheep erythrocytes (SRC), the agglutinin response to the latter eventually assumed all the characteristics of a T-independent response, i.e. several peaks of 19S antibody occurred with 10-day cycles before eventual slow 7S antibody production occurred (Fig. 5). This 19S anti-SRC cyclical type response could be converted to a single peak response, resembling the normal anti-SRC response, by an injection of 1 ml 7S anti-SRC antibody at 9 days (Fig. 6a and b). It is of interest that the passive administration of 7S anti-S. adelaide achieves the same effect. However, the reverse does not occur. As seen from Fig. 4c, passive 7S anti-SRC fails to modify the characteristics of the 19S antibody response to S. adelaide.

Clearly the normal immunological response to an infection involves an initial production of IgM antibody followed by IgG antibody, and on the basis of the foregoing arguments the appearance of the latter determines the decline of IgM antibody
The chronic antibody response

The past decade has witnessed the gradual build-up of an increasingly complicated battery of tests for Brucella antibody; direct agglutination, agglutination after addition of anti-human globulin, agglutination after 2-mercaptoethanol, and the complement fixation test. The results of all these have been used to deduce the presence of antibody which is IgM, IgG or IgA. This is only possible in certain instances since IgM, IgA and IgG all give rise to direct agglutination (Wilkinson, 1966), both IgM and IgG can achieve complement fixation, and the relative proportion of individual immunoglobulins which contribute to a titre may be obscure.

The basis of the radio-immunoassay (Nielsen, et al., 1973; Parratt et al., 1977) depends upon the use of a heavy suspension of Brucella organisms, which represents an excess of antigen which can absorb all of the specific Brucella antibody in 50 µl of serum. After adequate washing, an excess of ¹²⁵I-labelled sheep anti-human IgG (or IgM or IgA) is added. The amount of uptake of the latter, after washing, measures the antigen-combining power of the serum. Each anti-human Ig must be specific for one of the heavy chains of IgM, IgG or IgA. Also each serum must be tested for rheumatoid factor (RF) since if this is present it will elevate falsely the recorded IgM antibody. In the case of sera with positive RF, these can be absorbed before assay with aggregated human IgG.

In acute cases of brucellosis IgM antibody would be expected in the serum without much IgG or IgA antibody. If IgG has appeared in substantial amounts and produced negative feedback of the immunological response, the IgM antibody would be expected to fall to a low level, and it can be assumed that the chronic stage of a normally evolving immunological response has been reached. In Table 1, cases 1, 2 and 3 fit with the foregoing concept of acute brucellosis. Reactivity counts above 40 × 10⁶ are regarded as significant for the presence of antibody. Thus, case 2 with 3 weeks pyrexia has high IgM antibody with low IgG antibody. Presumably cases 1 and 3 are approaching the subacute phase of disease since they already have substantial levels of IgG antibody. Case 4 is clearly a chronic case with high IgG in the absence of IgM antibody. Similarly cases 5 and 6 clearly conform with a diagnosis of acute brucellosis and cases 7 and 8 with chronic brucellosis.

It should be noted that all the first 8 cases have some positive serological finding using conventional tests. How do the latter correlate with the results of the radio-assay? Firstly, how does radio-assay of IgM antibody correlate with direct agglutination titres? Of the forty-six sera examined only nineteen showed significant titres (1 : 80 or above) in the direct agglutination test. Only one serum showed a

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Fig. 5. Effect of an injection of 10⁶ Salmonella adelaidae on the serum antibody response, as detected by radio-assay of 19S antibody, to the simultaneous injection of 10⁹ sheep red cells. Adult fowl were injected at day 0 and serum antibody levels followed for 30 days. Note that the simultaneous injection of the bacillary antigen has modified the response to sheep red cells so that it fails to terminate at 15 days and goes through further cycles of 19S antibody production. O—O 19S antibody to SRC in chicken injected with SRC. ●—● 19S antibody to SRC in chickens injected with SRC+S. adelaidae. (Data from White and Nielsen, 1973.)

levels. But this IgM→IgG antibody switch can be delayed, e.g. by T-independent antigens and by an effect of T-independent antigens on the immune mechanism of T-dependent antigens. Decomplementation of the bird by treatment with cobra venom also delays the switch of IgM to IgG for the T-dependent SRBC (Nielsen and White, 1974; White and Nielsen, 1975).

The same type of radio-assay has been applied (Nielsen, Parratt and White, 1973) to the determination of the IgM, IgG and IgA types of antibody in human brucellosis. The assay has the great advantage over other methods of being a primary assay, i.e. it detects any antibody with the ability to combine with the surface antigens of Brucella organisms. It thus avoids all the distractions and complications of incomplete or blocking antibody, a notorious component of Brucella antisera.
negative radio-assay and a direct agglutination titre of 1:80. In general, there was a very poor correlation between the radio-assay for IgM antibody and the direct agglutination. Seventeen sera with direct agglutination titres < 1:80 had raised concentrations of IgM antibody by radio-assay. Secondly, the indirect agglutination results correlated moderately well with IgG antibody concentrations determined by radio-assay. Similarly, radio-assay levels for IgG antibody correlated well with the titres obtained by complement fixation.

In Table 1, numbers 8–22 represent cases in which all conventional serological tests were negative. With cases 9, 12, 13, 16 and 20, the radio-assay results agree with conventional tests and a diagnosis of brucellosis can be excluded. In case 10, the radio-assay results indicate acute brucellosis as suggested by the increased IgM antibody level. The findings of case 14 indicate chronic brucellosis and those of case 15 indicate a low level of residual antibody, although it is atypical to find that this is IgM antibody. In cases 17, 18 and 19 the diagnosis of brucellosis is supported, and radio-assay reveals antibody undetected by the conventional tests. Case 21 (Payne, 1974) is very interesting in that repeated conventional tests in several different laboratories proved negative in spite of the isolation of Brucella abortus from the blood. The serum radio-assay indicated high concentrations of IgM and IgG antibody which confirm the relapse of a chronic brucellosis infection. Case 22 yielded radio-assay results (high IgA antibody) which were compatible with a diagnosis of chronic brucellosis.

It remains to be determined whether this quantitative and sensitive primary assay for Brucella antibody will prove useful for the diagnosis of a relapse during the course of chronic brucellosis. This could be signalled by a change from a low IgG antibody level to a rising peak of IgM with later IgG rise. The significance of the results might be greatly increased by the contemporaneous testing of sequential serum samples.

With conventional tests the diagnosis of brucellosis could never be excluded, even if the agglutination test, the mercaptoethanol test, the complement fixation test and the anti-human globulin findings were all negative (Payne, 1974). The use of a sensitive radio-assay which covers the primary reactivity of Brucella antibody of IgM, IgG and IgA classes...
The chronic antibody response

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Clinical data</th>
<th>RIA ct/min \times 10^8</th>
<th>Direct agg. titre</th>
<th>Indirect agg. titre</th>
<th>CFT titre</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>IgM</td>
<td>IgG</td>
<td>IgA</td>
<td></td>
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<tr>
<td>1</td>
<td>Positive blood-culture of <em>B. abortus</em> 2 months previously</td>
<td>103</td>
<td>109</td>
<td>30</td>
<td>640</td>
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<tr>
<td>2</td>
<td>Pyrexia 3 weeks</td>
<td>68</td>
<td>34</td>
<td>6</td>
<td>160</td>
</tr>
<tr>
<td>3</td>
<td>Vet pricked by inoculation needle</td>
<td>71</td>
<td>65</td>
<td>8</td>
<td>&lt;20</td>
</tr>
<tr>
<td>4</td>
<td>Farmer with night sweats and pyrexia. Son with brucellosis</td>
<td>4</td>
<td>140</td>
<td>10</td>
<td>5120</td>
</tr>
<tr>
<td>5</td>
<td>Pyrexia with no obvious cause, of 2 weeks' duration</td>
<td>200</td>
<td>138</td>
<td>47</td>
<td>1280</td>
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<td>6</td>
<td>Dairy farmer: pyrexia 2 weeks. Rash and joint pains</td>
<td>164</td>
<td>0</td>
<td>0</td>
<td>80</td>
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<td>7</td>
<td>Slaughterman with backache and febrile episodes</td>
<td>26</td>
<td>63</td>
<td>0</td>
<td>160</td>
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<tr>
<td>8</td>
<td>Malaise, giddiness, nausea, headache, and depression</td>
<td>27</td>
<td>63</td>
<td>0</td>
<td>1280</td>
</tr>
<tr>
<td>9</td>
<td>Farmer: chronic ill health</td>
<td>4</td>
<td>13</td>
<td>0</td>
<td>&lt;20</td>
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<tr>
<td>10</td>
<td>Farm dweller: intake of raw milk; painful hip</td>
<td>82</td>
<td>0</td>
<td>0</td>
<td>&lt;20</td>
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<td>11</td>
<td>Farmer's wife: pyrexia of uncertain cause: rigors and backache</td>
<td>53</td>
<td>0</td>
<td>14</td>
<td>&lt;20</td>
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<tr>
<td>12</td>
<td>Veterinary staff: routine check</td>
<td>0</td>
<td>7</td>
<td>0</td>
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</tr>
<tr>
<td>13</td>
<td>Veterinary staff: routine check</td>
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<td>29</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>14</td>
<td>Farmer with recurrent fever</td>
<td>24</td>
<td>15</td>
<td>58</td>
<td>&lt;20</td>
</tr>
<tr>
<td>15</td>
<td>Recovered from brucellosis</td>
<td>55</td>
<td>0</td>
<td>0</td>
<td>40</td>
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<tr>
<td>16</td>
<td>Farmer: history of aches and pains</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>17</td>
<td>Cowman: history of multiple complaints</td>
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<td>75</td>
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<td>18</td>
<td>Recurrent pyrexia</td>
<td>65</td>
<td>33</td>
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<td>&lt;20</td>
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<td>19</td>
<td>Chronic brucellosis</td>
<td>113</td>
<td>28</td>
<td>0</td>
<td>80</td>
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<tr>
<td>20</td>
<td>Farmer with 3rd episode of pyrexia of unknown origin</td>
<td>0</td>
<td>0</td>
<td>63</td>
<td>&lt;20</td>
</tr>
<tr>
<td>21</td>
<td><em>Brucella</em> sp. isolated from blood 1966. Negative serology. Several relapses. Possibly new relapse</td>
<td>84</td>
<td>70</td>
<td>0</td>
<td>&lt;20</td>
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<td>22</td>
<td>Herd manageress: recurrent fever</td>
<td>3</td>
<td>42</td>
<td>89</td>
<td>&lt;20</td>
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</table>

should go a long way towards allowing the exclusion of a brucellosis diagnosis – always provided that the patient is not incapacitated by a severe immunological deficiency.

References


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doi: 10.1136/pgmj.54.635.595

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