SELECTIVE IMMUNOGLOBULIN DEFICIENCY IN CATTLE AND SUSCEPTIBILITY TO INFECTION

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Sera from animals, mainly of the Red Danish Milkbreed, have been examined by immunochemical analyses in order to select possible IgG-2 immunoglobulin deficiencies. According to previous findings a low frequency was found in the healthy animal material (1 out of 417 animals) whereas the frequency of this immunoglobulin deficiency was strikingly much higher among diseased animals, especially among animals with pyogenic infections (13 out of 93 animals). It was assumed that lack of IgG-2 may be associated with a reduced resistance to infections, in particular those caused by pyogenic bacteria.

Four classes of immunoglobulins have been identified in cattle, IgG-1 and IgG-2 have been described and characterized in detail by Murphy et al. (1965), Pierce and Feinstein (1965) and Kickhöfen et al. (1968). The existence of IgM (Murphy et al. 1965) and IgA (Mach et al. 1969, Vaerrman 1970) have likewise been demonstrated. A series of experiments, most recently by Klaus et al. (1969), have shown that IgG-1 is selectively transferred from serum to colostrum. Hence, the post-suckling calf serum usually contains substantial quantities of this immunoglobulin class, passively derived from the dam. Gradually, this maternally derived immunoglobulin is catabolized and successively the synthesis of all classes of immunoglobulins is initiated under the influence of environmental antigenic stimulation and increasing immunological competence. Consequently, immunoglobulin deficiency may be based upon one or more of the following circumstances: 1) Lack of maternal immunoglobulin in the new-born, 2) failure of the young animal to initiate synthesis and 3) discontinuation of an existing synthesis. While much attention has been attached to neonatal immunoglobulin deficiency secondary to inadequate colostral intake or failure to absorb immunoglobulin from the gut, there seems to be only scanty information available on lack of immunoglobulins in adult cattle.

Mansa (1965) described a selective immunoglobulin deficiency in normal adult cattle of the Red Danish Milkbreed. By immunoelectrophoretic analysis it was found that 8 out of 780 adult animals apparently lacked an immunoglobulin component, designated 7S-gamma. When comparison is made with studies by Murphy et al. (1965) and Aalund (1968) it appears that Mansa’s 7S-gamma globulin is identical with IgG-2. Furthermore, lack of immunoglobulin in adult cattle has been reported from Central Africa (Provost...
et al. 1965). By immunoelectrophoresis it was demonstrated that a small but not precisely determined number of animals lacked a cathodically migrating immunoglobulin which presumably corresponded to IgG-2.

In a recent study comprising 29 adult cattle patients with various diseases, including bacterial and parasitic infections 5 animals (17 per cent) were deficient of IgG-2 (Nansen 1970). This contrasted with the finding that only 4 out of 180 normal sera (appr. 2 per cent) lacked this immunoglobulin. This apparent accumulation of immunoglobulin deficiency among diseased animals has been examined more closely in the present investigation.

**MATERIALS**

**Healthy Animals:**

417 sera were obtained from animals originating from two different geographical areas.

a) During the years 1968–1969, 320 sera were obtained from normal female animals from eastern and southern regions of Jutland. Ages of the animals ranged from 3 to 12 years, the average age being 6 years. Distribution of animals on breeds was as follows: 21 per cent belonged to the Black Pied Danish Milkbreed (SDM), 59 per cent belonged to the Red Danish Milkbreed (RDM), 19 per cent were Jerseys and 1 per cent were crossbreeds.

b) In 1968 serum samples were obtained from 97 normal female animals from various herds of Sealand. Ages ranged from 1 to 9 years, averaging 4 years. There were 93 per cent RDM’s and 7 per cent Jerseys.

**Diseased Animals:**

312 sera, including the above-mentioned 29 sera (Nansen 1970), were obtained from patients housed in the Medical Clinic for Large Domestic Animals during the years 1967–1969. The majority of animals originated from herds at Seeland. The patient material of the clinic does not reflect entirely the disease situation in the area of Seeland as a whole, since most admitted animals suffer from chronic diseases with a rather high lethality. At approximately monthly intervals, serum was drawn routinely from all hospitalized animals more than six months old.

The collected samples were not representative of the entire patient material, since animals with acute, fatal diseases or animals with mild, easily curable diseases stayed in the clinic for only short periods and consequently were not always subject to the routine blood sampling. The collected sera originated from appr. 50 per cent of the patients in the age group ½ to 9 years. The average age was 5 years. Seventyfive per cent were RDM’s, 13 per cent were Jerseys, 8 per cent were SDM’s and 4 per cent of the animals were other breeds or crossbreeds. The vast majority of animals were females. Thus, there were only 13 bulls (11 RDM’s, 1 SDM and 1 crossbreed). According to the aetiology of the disease the animals could be classified into 4 groups: A: 100 animals with noninfectious diseases like, e.g. intoxications, nutritional and metabolic disorders, B: 89 animals with viral, parasitic or bacterial diseases other than pyogenic bacterial infections, C: 30 animals with pyogenic bacterial infections secondary to traumatic lesions, e.g. traumatic reticuloperitonitis, teat lesions, surgical incisions etc., D: 93 animals with primary pyogenic bacterial infections, e.g. purulent bronchopneumonia, mastitis, endometritis, pyaemia.

The sera were stored in small aliquots at minus 20°C until immunoelectrophoretic analysis.

**METHODS**

All sera were screened by immunoelectrophoresis using the micromethod of Scheidegger (1955). Antisera were obtained from rabbits immunized with pooled serum from 22 normal animals (RDM). Examination of serial dilutions of IgG-2 demonstrated that visible precipitates were formed in immunoelectrophoresis with IgG-2 concentrations down to approximately 180 µg/ml. When no IgG-2 precipitates were formed in the immunoelectropherograms, the sera were further analyzed by single radial immunodiffusion according to the method of Mancini et al. (1965) as slightly modified by Jensen (1966). The antisera imbedded in the agar gel was obtained by immunizing rabbits with IgG-2 isolated from one serum (RDM) by anion-exchange chromatography on DEAE-Sephadex. Monospecificity was achieved by absorption of the rabbit antiserum with IgG-2 deficient serum. Application of serial dilutions of IgG-2 to 4 different agar plates revealed that the lowest concentration which produced visible precipitates was 6 µg/ml. This limit could not be lowered further since a reduction of the antibody content in the agar produced indistinct precipitates. Thus, the present procedure would distinguish sera with IgG-2 concentrations below 6 µg/ml from sera with IgG-2 levels in the range of 6 to 180 µg/ml.

**RESULTS**

**Healthy Animals:**

Among the 320 animals from the Jutland area, one RDM cow (6 years) was deficient.
of IgG-2 as evidenced by immunoelectrophoresis and single radial immunodiffusion. This animal was clinically normal and presented no history of e.g. recurrent infections. All animals from the Sealand area contained IgG-2 as detected by immunoelectrophoresis.

**Diseased Animals:**

Among the 312 patients examined, IgG-2 could not be demonstrated by immunoelectrophoresis (Fig. 1) in 22 female animals (7 per cent). All these animals belonged to the Red Danish Milkbreed. Ages and main diagnoses are listed in Table 1. The IgG-2 deficiency was very unequally distributed among the 4 major disease groups. Thus, only one out of 100 animals of the non-infectious group A was IgG-2 deficient (1 per cent) while 5 out of 89 animals of group B (6 per cent) and 3 out of 30 animals of group C (10 per cent) lacked this immunoglobulin. The highest number of IgG-2 deficient animals was found in the group of animals with pyogenic infections (group D), i.e. 13 out of 93 animals (14 per cent). In only 4 animals, one of group A, two of group C and one of group D, could IgG-2 be demonstrated by the more sensitive single radial immunodiffusion analysis (see Table 1). Thus, the majority of the selected immunoglobulin deficient sera contained less than 6 µg IgG-2 per ml. Ages of the IgG-2 deficient animals (average 3 years) were somewhat lower than those of animals in the entire patient material (average age: 5 years). While the IgG-2 deficient animals of groups A, B and C were admitted to the clinic with very different anamneses, the immunoglobulin deficient animals of group D seemed to share a more characteristic disease history.

**Table 1. Ages and Diagnoses of 22 IgG-2 Deficient Female Animals of the Red Danish Milkbreed**

<table>
<thead>
<tr>
<th>Animal J. No.</th>
<th>Age (years)</th>
<th>Diagnosis</th>
<th>Disease group</th>
</tr>
</thead>
<tbody>
<tr>
<td>138/66</td>
<td>2</td>
<td>Abomasenteritis catarrhalis</td>
<td>B</td>
</tr>
<tr>
<td>187/66</td>
<td>2½</td>
<td>Bronchopneumonia purulenta et necroticans</td>
<td>D</td>
</tr>
<tr>
<td>217/66</td>
<td>5</td>
<td>Enteritis paratuberculosa</td>
<td>B</td>
</tr>
<tr>
<td>306/66</td>
<td>2</td>
<td>Peritonitis fibrosa et purulenta traumatica</td>
<td>C</td>
</tr>
<tr>
<td>314/66§</td>
<td>2</td>
<td>Bronchopneumonia purulenta</td>
<td>D</td>
</tr>
<tr>
<td>374/66</td>
<td>1</td>
<td>Bronchopneumonia purulenta</td>
<td>D</td>
</tr>
<tr>
<td>389/66</td>
<td>3</td>
<td>Bronchopneumonia purulenta apostematosa</td>
<td>D</td>
</tr>
<tr>
<td>420/66</td>
<td>3</td>
<td>Bronchopneumonia purulenta</td>
<td>D</td>
</tr>
<tr>
<td>449/66§</td>
<td>3</td>
<td>Encephalomalacia</td>
<td>A</td>
</tr>
<tr>
<td>60/67</td>
<td>1</td>
<td>Abomasitis hyperplastica verminosa</td>
<td>B</td>
</tr>
<tr>
<td>189/67</td>
<td>2</td>
<td>Bronchopneumonia purulenta, Lymphocytosis</td>
<td>D</td>
</tr>
<tr>
<td>194/67</td>
<td>3</td>
<td>Abomasenteritis catarrhalis</td>
<td>B</td>
</tr>
<tr>
<td>274/67</td>
<td>5–7</td>
<td>Mastitis purulenta</td>
<td>D</td>
</tr>
<tr>
<td>278/67§</td>
<td>3</td>
<td>Peritonitis purulenta traumatica</td>
<td>C</td>
</tr>
<tr>
<td>290/67</td>
<td>6–7</td>
<td>Pyaemia</td>
<td>D</td>
</tr>
<tr>
<td>375/67</td>
<td>2</td>
<td>Fascioliasis</td>
<td>B</td>
</tr>
<tr>
<td>398/67</td>
<td>2</td>
<td>Bronchopneumonia purulenta</td>
<td>D</td>
</tr>
<tr>
<td>446/67</td>
<td>2½</td>
<td>Pyaemia</td>
<td>D</td>
</tr>
<tr>
<td>59/68</td>
<td>2½</td>
<td>Bronchopneumonia purulenta</td>
<td>D</td>
</tr>
<tr>
<td>420/68</td>
<td>3</td>
<td>Pyaemia</td>
<td>D</td>
</tr>
<tr>
<td>423/68</td>
<td>2½</td>
<td>Bronchopneumonia purulenta</td>
<td>D</td>
</tr>
<tr>
<td>487/68§</td>
<td>2½</td>
<td>Peritonitis purulenta traumatica</td>
<td>C</td>
</tr>
</tbody>
</table>

§ IgG-2 demonstrable by the subsequent radial immunodiffusion analysis.
Before these animals arrived to the clinic there had usually been periods of repeated attacks of infection, the first of which often coincided with the postparturient period. The infections might be temporarily controlled by antibiotics but recurred after cessation of treatment. The same pattern was established during the stay in the clinic where the general condition of the animals usually would be very poor. The majority of animals were killed after some weeks of observation. *Corynebacterium pyogenes* was the bacterium most frequently isolated. Also *Streptococcus-* and *Micrococcus pyogenes* were isolated from some animals. It is noticeable that the formol-gel reaction was negative in 7 out of the 13 IgG-2 deficient animals with pyogenic infections.

Finally, it should be mentioned that IgG-1 was always demonstrable by immunoelectrophoresis both in normal and diseased animals. IgM and IgA precipitation lines, which may sometimes be difficult to recognize and identify in the immunoelectropherograms, were not evaluated.

**DISCUSSION**

The lack of detectable serum IgG-2 is assumed to be due to an extremely reduced or even failing synthesis, and not to excessive catabolism (Nansen 1970). If an individual is not synthesizing a protein present in other members of the same species, *e.g.* due to lacking genetic information, immunization with this protein may induce the synthesis of iso-antibodies. Conversely, such iso-antibodies may be indirect proof of a complete and genuine lack of the protein concerned. Gahne (1964) was able to develop iso-antibodies against bovine alpha-2 macroglobulin by injecting normal serum into a cow with an inhereted lack of this protein. Similarly, attention should be focused on the possible production of iso-antibodies against bovine IgG-2. It should be emphasized that a genetic basis for many dysimmunoglobulinaemic conditions of man has been demonstrated and the question arises whether lack of IgG-2 in cattle might have a similar background. Unfortunately, available information about this very important point is still insufficient and inconclusive. The distribution between males and females should be closely examined in this context. IgG-2 deficient bulls were not found in the present study comprising 13 bulls, and 280 sera from healthy adult bulls (RDM's mainly) all contained IgG-2 (Mansa 1970). A larger number of males should be examined to reveal possible IgG-2 deficiencies.

Observations by the author (Nansen 1970) and by Aalund and Kruse (1971) seem to indicate the existence of at least two antigenic entities within IgG-2. Presumably, not all sera contain the entire IgG-2 antigenic spectrum. Since IgG-2 used for immunization in the present study was isolated from one serum only, the rabbit antiserum might be incomplete in the sense that it could not detect all IgG-2 antigenic determinants. The antiserum used for the initial immunoelectrophoretic screening, on the other hand, was developed by immunizing rabbits with a pool of serum from several animals. Sera which did not develop IgG-2 precipitates with this antiserum were submitted to immunoquantitation in agar gel containing the above-mentioned anti-IgG-2. It should therefore be emphasized that IgG-2 components which might remain unrecognized with the used anti-IgG-2 occurred at concentrations too low to be detected by immunoelectrophoresis using a more “complete” antiserum.

The most conspicuous finding in the present study was the uneven distribution of nonetectable levels of IgG-2 between normal sera and sera obtained from diseased animals. The low frequency found in the normal material (1 out of 417 animals) corresponded to previous findings (Mansa 1965, 1970, Nansen 1970) whereas the frequency among the diseased animals was strikingly much higher, especially among animals with pyogenic infections. This leads to the assumption that lack of IgG-2 is associated with a reduced resistance to certain infections, especially to those caused by pyogenic
bacteria. A particular role of IgG-2 in the defence against pyogenic bacteria could be expected. Thus, these infections are usually associated with an increased production of especially IgG-2 in animals with normal ability to synthesize immunoglobulins (Nansen 1970), and generally the course of infections in the latter animals was not as severe as that in IgG-2 deficient animals. An alternative possibility is that the hospitalized animals have predominantly been admitted from certain cattle herds or families with a genetically determined high frequency of non-detectable levels of IgG-2. However, this could only play a minor role, if any, since 1) the animals originated from a variety of herds in different areas of Sealand and 2) no more than one IgG-2 deficient animal was admitted from any herd and 3) all 100 patients but one with non-infectious diseases (group A) contained IgG-2 in their sera.

Immunoglobulin deficiency syndromes in man have been classified into several groups, most of which can be recognized only when the individual classes or subclasses can be identified as, e.g. by immunoelectrophoresis or specific immunoquantitation. In case the synthesis of one or possibly two immunoglobulin classes is disturbed the term dysimmunoglobulinaemia has been proposed (Zawadski & Edwards 1967), and lack of IgG-2 in cattle can be designated accordingly. Numerous dysimmunoglobulinaemias due to failing synthesis in man have been described (Hobbs et al. 1967, Pelkonen 1969, Rådl 1970). They constitute a heterogeneous group of syndromes designated primarily by the class or classes of immunoglobulins involved. It appears that most selective immunoglobulin deficiencies in man are associated with decreased resistance to infections. In addition, many of them are associated with various disorders such as defects of the thymus system, malabsorption syndromes and ataxia telangiectasias. Apparently, the lack of bovine IgG-2 was primarily characterized by decreased resistance to infections and not associated with any obvious syndromes. Although the immunoglobulin deficiency in the bovine is selective, it offers clinical points of similarity with the congenital, sex-linked "broadbanded" hypoinmunoglobulinaemia in man, usually referred to as the "Bruton type" (Bruton 1952). Thus, a typical feature of this syndrome is the reduced resistance to pyogenic bacteria such as micrococci, streptococci and meningococci. Usually, the first symptoms are seen at ages of 6 months to 3 years, which is comparable with the observation in the present study that IgG-2 deficient patients were relatively young animals. Lung infections are most frequently observed in the "Bruton type" of hypoinmunoglobulinaemia, but otitis, sinuitis and septicaemia are often encountered. Like in IgG-2 deficient cattle, the infections may be temporarily controlled by antibiotics, but recurrence is a nearly unavoidable feature. On the other hand development of viral infections are apparently not more severe than those in normal individuals. This appears to be in accordance with findings in studies by Provost et al. (1965) who observed that immunoglobulin (IgG-2?) deficient cattle in Central Africa were not more susceptible than normal animals to viral diseases such as Rinderpest, despite the fact that the immunoglobulin deficiency coincided with a lack of in vitro detectable specific antibodies. Finally, it should be noted that Leptospira agglutinins were found to be evenly distributed among normal and IgG-2 deficient sera of the Red Danish Milkbreed (Mansa 1965).

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