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Immunodeficiency in the Chicken

I. DISPARITY IN SUPPRESSION OF ANTIBODY RESPONSES TO VARIOUS ANTIGENS FOLLOWING SURGICAL BURSECTOMY

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Summary. The extent of suppression of antibody response by bursectomy (Bx) was examined as a measure of the seeding sequence of different clones from the bursa to peripheral lymphoid tissues. Chickens were bursectomized surgically 1, 4 or 7 days after hatching and immunized later with four antigens: sheep red blood cells (SRBC); *Bordetella pertussis* (*Bp*); human serum albumin (HSA); influenza virus (IV). The kinetics of the antibody responses were delayed in bursectomized birds when compared with the control groups. The following order in the degree of immunosuppression was established: *Bp*>HSA>SRBC>IV. This is discussed in relation to the 'sequential maturation' theory of ontogenesis of immunocyte differentiation. The data also stress the limitation of non-specific markers for assessing partial immunodeficiency states.

INTRODUCTION

A distinct maturation sequence of immune responsiveness to different antigens has been described throughout the gestation period of the foetal lamb. This sequence was interpreted as contradictory to the somatic mutation theory for generation of antibody diversity (Silverstein and Prendergast, 1970). It is not known, however, whether the onset of antibody responsiveness during early ontogeny reflects the maturation pattern of B cells or of other limiting factors, such as T cells (Carter and Rector, 1972; Chiscon and Golub, 1972; Arrenbrecht, 1973), macrophages (Argyris, 1968) or material antibody (Leiper, 1974). Adoptive transfer of cells of an individual spleen colony (progeny of a single stem cell) restores competence towards flagellin and SRBC in 20 and 30 days respectively (Yung, Wyn-Evans and Diener, 1973). This is compatible with Jerne's theory of somatic mutation, according to which approximately 20 days would be required for the generation of B lymphocytes recognizing a panel of 10,000 antigens. Other authors, however, have drawn conflicting conclusions from demonstrations of almost simultaneous appearance of antigen-binding cells and antibody responsiveness to at least two different antigens (Spear, Wang, Rutishauer and Edelman, 1973).

It appeared to us that the 'sequential maturation' hypothesis could be investigated with advantage in chickens, whose B cells are generated in the bursa of Fabricius and seeded

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into peripheral tissues shortly before and after hatching. Surgical bursectomy at various days post-hatching arrests this peripheralization and immunization at a later age should reflect the commitment of the limited pool of cells which had seeded prior to bursectomy (Ivanyi, 1973). The influence of other limiting factors (T cells, macrophages, natural antibody) which may play a role if animals are immunized perinatally is thereby effectively excluded, for surgical bursectomy impairs the development of the B-lymphoid system specifically. We assumed that the relative suppression of antibody responsiveness to various antigens by bursectomy performed at different times post-hatching would reflect the seeding sequence of various B-cell clones.

MATERIALS AND METHODS

Chickens

Brown leghorn outbred chickens were hatched from eggs laid by a specific pathogen-free flock. The 22nd day of incubation corresponded to the 1st day post-hatching. Surgical bursectomy (Bx) was performed under ether anaesthesia and 0·05 per cent aureomycin was added to the drinking water for 14 days. Subsequent gain in body weight of bursectomized birds did not differ from that of controls.

Antigens and immunizations

Sheep erythrocytes (SRBC) (Wellcome Reagents) were washed in saline and injected at a dose of 10^{10}. A suspension of formalin-killed *Bordetella pertussis* organisms (*Bp*), prepared and kindly donated by Dr P. Novotny (Department of Bacteriology) was used at a dose of 1 ml (1 mg dry weight). Influenza virus (IV) (Admune Mono from BDH) was used at 0·7 ml suspension dose. Human serum albumin (HSA) (SEVAC, Prague) was used at a dose of 5 mg. All antigens were injected intravenously in doses related to 1 kg body weight.

Serological assays

Haemagglutination (HA) of SRBC and of SRBC sensitized with HSA by *bis*-diazotized benzidine was performed using the Takatsy microdilution technique, recording $\log_2$ values of end point dilutions (Ivanyi, Valentova and Cerny, 1966). Agglutination reactions with *Bp* cells were read under a dissecting microscope following incubation overnight at 37°. Antibodies against IV were quantificated by haemagglutination inhibition (HI). Serially diluted sera were incubated for 15 minutes at room temperature with IV at a dilution of two $\log_2$ values below its agglutinating titre. Subsequently, a 1 per cent suspension of washed chicken erythrocytes was added and the HI-titre was read 1 hour later. The chicken red cells bled from a standard panel of birds were obtained from the Department of Virology.

Treatment with 2-mercaptoethanol (2-ME) was performed by diluting serially the tested sera into 0·2 M 2-ME (instead of saline which was used as control), and incubating for 1 hour at 37° before adding the suspension of SRBC, *Bp* or IV. The 2-ME-sensitive titre was calculated by subtracting the titre in the presence of 2-ME from the titre in saline. Previous studies demonstrated that the sensitivity towards treatment with ME in this system is an adequate criterion distinguishing the majority of IgM and IgG type of antibodies (Ivanyi *et al.*, 1966). This was confirmed again in this study by analysing representative sera by sucrose-gradient centrifugation with subsequent serological assay of 19S and 7S fractions.

All serological assays were performed within 1 week of bleeding with sera stored at 4°. Duplicate samples were assayed and arithmetic means ± standard error (s.e.) values for the experimental groups were calculated by a computer programme.

Quantitative determination of the serum IgG level was performed by the radial diffusion method of Mancini, Carbonara and Heremans (1965).

RESULTS

The serum IgG level of chickens surgically bursectomized 1, 4, or 7 days post-hatching was partially suppressed (Table 1). Whether all B-cell clones or only those committed to a few antigens were impaired was examined by determining the humoral response to immunization by four randomly selected antigens of widely different nature.

Simultaneous primary immunization of 1-day Bx chickens with SRBC and *Bp* resulted in a reduced IgM response to SRBC and a complete lack of response towards *Bp* (Fig. 1). However, 4-day Bx chickens showed a delayed IgM response to SRBC, but of the same

TABLE 1

SERUM IgG CONCENTRATION IN SURGICALLY BURSECTOMIZED BIRDS*

Bursectomy day post-hatching	Number of birds	IgG (mg/ml of serum)	
		Mean	±s.e.
1	16	1·02	·16
4	18	1·18	0·20
7	16	1·40	0·12
None	17	2·16	0·46

* Chickens aged 6 weeks.

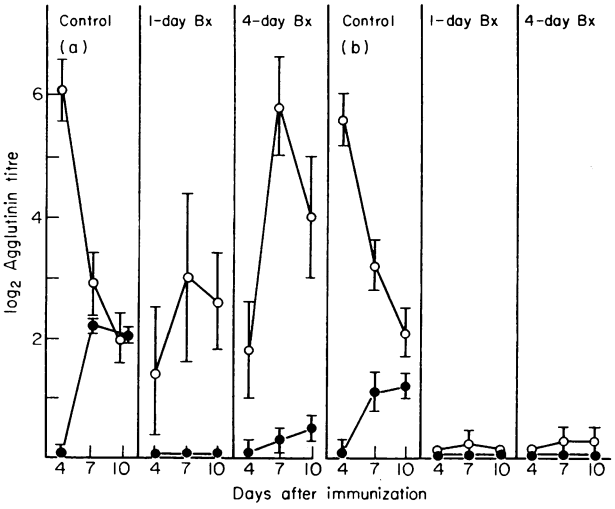

FIG. 1. Primary responses to (a) sheep red blood cells and (b) *Bordetella pertussis*. Mean values from six to nine chickens per group immunized simultaneously with 10^{10} SRBC and 1 mg BP at the age of 7 weeks. (○) IgM. (●) IgG.

peak titre as controls, while antibodies against *Bp* were practically absent. The rapid decline of anti-SRBC antibodies in the control group is due apparently to the rise and feedback effect of IgG antibodies which were absent in the Bx groups. Some Bx chickens manifested IgM anti-SRBC HA titres higher than the corresponding controls which accounts for the large standard error of the mean values.

The antibody responses to IV and HSA are compared in Fig. 2. The majority of the HI-antibody titre against IV was 2-ME-resistant and sucrose-gradient analysis confirmed its 7S nature. The antibody titre declined slightly in the control but increased in the 4-day Bx group between the 4th and 10th day post immunization. Apart from these inverse kinetics, there was no quantitative difference in the peak titre of control and Bx groups. However, bursectomy resulted in a contrasting pattern of responses to HSA. 2-ME-sensitive (IgM) antibodies were reduced in the 4-day Bx groups and in the 7-day Bx groups showed a delayed (10-day) peak titre similar to the early (4-day) response of controls. The 2-ME-resistant (IgG) response was severely inhibited in both 4-day and 7-day Bx groups. These results confirm recent and more extensive data which demonstrated furthermore an anamnestic IgG response to HSA in 7-day but not in 4-day bursectomized chickens (Ivanyi, 1973).

DISCUSSION

We have demonstrated that chickens with partial deletion of their B cells manifested contrasting degrees of inhibition of the primary response to four randomly selected antigens. Nevertheless, a number of points must be carefully considered before accepting that the data unequivocally support the 'sequential maturation' hypothesis (Fig. 3).

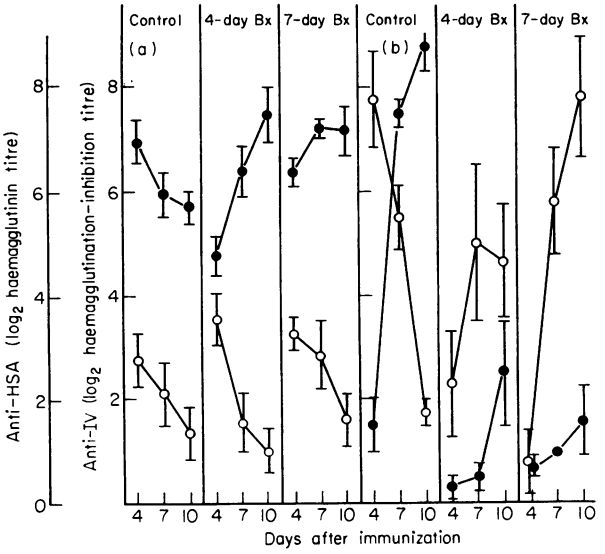

FIG. 2. Primary responses to (a) influenza virus and (b) human serum albumin. Mean values from six to nine chickens per group immunized with 0·7 ml of IV at 8 weeks and with 5 mg of HSA at 16 weeks. (○) IgM. (●) IgG.

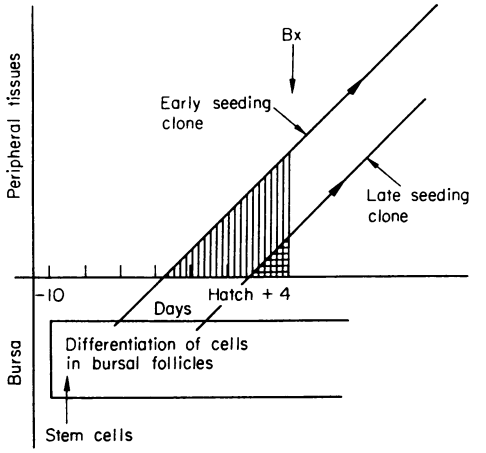

FIG. 3. Schematic diagram of the 'sequential maturation' hypothesis for B-cell ontogeny in the chicken. Shaded areas represent the fractions of the respective clones which peripheralized by the time of bursectomy.

Agglutination methods used in this study were probably of a similar order of sensitivity. Moreover, differences in sensitivity of quantitation of antibodies to the four antigens tested would not have influenced the conclusions since the relative antibody suppression towards a particular antigen was determined by comparison with the average titre of the control group (which was sufficiently high in respect to all four antigens). This is also taken as evidence that optimal or near-optimal antigen doses were chosen.

Antibody levels at the peak of the primary antibody response are widely used as a measure of the size of an antigen-reactive cell clone (Makinodan and Albright, 1967). The evaluation of the 'peak antibody response', however, represents the main obstacle for quantitative extrapolation of the present data. Bursectomized chickens produced a delayed response which reached a late peak of antibody level sometimes approaching that of the control group; in other instances, however, only marginal or no recovery was observed (anti-*Bp*, IgG anti-HSA). Although there is little doubt that the anti-SRBC response was less suppressed when compared with *Bp*, it is difficult to suggest a representative numerical figure in view of the shifted time course of the response. It has been previously established that all antigen-sensitive cells are not triggered by antigen simultaneously but recruited from different cellular compartments and induced asynchronously into proliferation and antibody synthesis (Ford and Gowans, 1967). Assuming that the clone of B cells committed to a certain antigen is composed of cells at different stages of maturation and/or anatomical distribution (Laufler, Miller and Phillips, 1972), it is likely that their proportional representation in the partially deficient Bx chicken would be changed with the 'mature' antigen-reactive cell types being more deficient. Neonatal B cells produce foci which secrete less than half the antibody of that secreted by foci originating from adult precursor cells (Press and Klinman, 1973), which supports our contention that, in addition to numerical depletion, the B cells of Bx birds might be functionally different and reminiscent of B cells present normally in neonatal animals. Another mechanism which probably contributes to the gradual rise and slow decline of antibody formation in Bx birds is the ineffectiveness of regulatory feedback suppression by IgG

antibodies which has previously been shown to be selectively suppressed and of low avidity (Ivanyi, 1973).

The preceding arguments suggest that B-cell clones committed to different antigens showed disparate degrees of impairment at the time of primary immunization. The question arises as to whether or not this is generated by true differences in the sequence of maturation at the bursal stage. Previous studies have suggested that the maturation of cells in the bursa and their subsequent output is not influenced by antigens (Kincade and Cooper, 1971; Ivanyi and Shand, 1973) which excludes an environmental influence on a predetermined maturation sequence. However, we considered the possibility that the limited clone of peripheralized cells expanded to different degrees as a result of cross-stimulation by environmental antigens. This argument, however, is not supported by our observation of the slow time-course of the antibody response—this being typical for primary responses. The high degree of suppression of the *Bp* response (as a common bacterial antigen) by late bursectomy would not be expected if environmental factors contributed to the expansion of peripheralized clones. On the other hand, there was a good chance that the birds had been in contact with and were sensitized by a human strain of influenza virus (non-pathogenic for chickens) which may have accounted for the predominantly IgG type of the response. It is feasible to assume also that there are considerable differences in the number and/or size of cell clones committed to respond to antigens or epitopes of various 'strength' and complexity. If clones are seeded randomly, there would be a greater probability of reaching limiting cell concentrations and full depletion of responsiveness towards less complex or 'weaker' antigens. Thus, we conclude that our results, although apparently supportive, do not provide decisive evidence in favour of the 'sequential maturation' hypothesis.

Finally, we wish to mention a practical implication of our observations in relation to assessment of human immunodeficiency diseases. Our previous finding of depressed antibody response in bursectomized chickens with normal lymphocyte transformation induced by anti-Ig serum *in vitro* (Ivanyi *et al.*, 1969) together with the present results, suggest that non-specific criteria of B-cell function or Ig synthesis are inadequate for determining the status of an individual with partial immunodeficiency: the immune response may be severely impaired to some antigens while near normal to others.

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