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Ontogeny of Haemolytic Plaque-Forming Cells in the Hamster; the Response to Sheep and Mouse Erythrocytes

J. B. SOLOMON, J. LEIPER AND T. M. S. REID

Department of Bacteriology, University of Aberdeen, Scotland

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Summary. The onset of ability to produce haemolytic antibody to sheep erythrocytes does not appear in hamsters until 11 days after birth. However, when mouse erythrocytes were used as antigen an earlier onset of immunocompetence at 6 days after birth was detected. The hamster can make haemolytic antibody only 9·5 days after the appearance of lymphocytes in the thymus—this is sooner than any other species. This capacity is compared with other species on the basis of physiological development by age-equivalence.

INTRODUCTION

The hamster is unique among mammals because of its short gestation period of 16 days; it is born only 3·5 days after lymphocytes are first seen in the thymus (Boyer, 1953; Weakley, Patt and Shepro, 1964). Predictions of the time of onset of immunocompetence from the age-equivalence theory (Solomon, 1970, 1971a) indicate that the hamster should respond to antigenic stimulation as soon as 3 days after birth. This would require a very rapid emigration of small lymphocytes from the thymus and bone marrow to the peripheral lymphoid organs. Sheep erythrocyte antigens have not been found to be very suitable in the present studies because of a rapid onset of 'natural' plaque-forming cells (PFC). On the other hand, the use of mouse erythrocytes as antigen has enabled a very clear-cut onset of the PFC response to be detected within 6 days after birth.

MATERIALS AND METHODS

Litters were obtained from our breeding colony of Syrian hamsters and weaned at 21 days of age. The number of offspring averaged four per litter. Mouse erythrocytes, for use as antigen or target cells, were collected fresh daily by cardiac puncture and kept in Alsever's solution. Sheep erythrocytes were obtained fortnightly from British Drug Houses Ltd. Immediately before they were required, erythrocytes were washed three times with ice-cold normal saline and the number of packed cells determined by haemocytometer count. Hamsters were injected intraperitoneally with a suspension of erythrocytes in normal saline (3×10^{10} cells/kg body weight). On this basis, a dose for a 2-day-old hamster was 8×10^7 cells and an adult received 4×10^9 erythrocytes. In order to avoid leakage of erythrocytes following the usual method for intraperitoneal injection, young hamsters up to 2 weeks of age were injected into the top (and slightly to the side) of the peritoneal cavity with the needle pointing towards the opposite hind leg.

Four days after immunization, spleens were removed from animals under aseptic conditions, cleared of adherent fat, and placed in Petri dishes containing Eisen's B solution (Helmreich, Kern and Eisen, 1961). The use of Eisen's B or C solutions has been recommended by Hübner and Gengozian (1969) because they enhance the complement activity of guinea-pig serum. Pilot tests indicated that Eisen's B solution was somewhat superior in this respect so this suspending medium was used throughout the following experiments. Spleens from each litter were pooled, whereas adult hamster spleens were prepared separately. Spleens were macerated through a nylon sieve and gently sucked through 21-gauge then 25-gauge needles to disperse any clumps. Any connective tissue was allowed to settle for a few minutes and the supernatant cells filtered through a double layer of Kleenex paper tissue. The cell suspension was centrifuged at 2,500 rev/min and the pellet washed twice with Eisen's B solution before final suspension. An aliquot of this suspension was added to Eosin Y solution (0·1 per cent w/v in Eisen's B solution) and counted in a haemocytometer. Eosin Y stains dead cells orange; we never found more than 10 per cent such cells in our spleen cell suspensions.

The method for detection of PFC described by Cunningham and Szenberg (1968) was adopted with some minor modifications. Erythrocytes ($3{\cdot}2 \times 10^8$) and spleen cells (usually 2×10^7) in suspension were diluted to 0·4 ml with Eisen's B solution. The number of nucleated spleen cells per slide must not exceed 4×10^6 or inhibition of plaque formation occurs. Guinea-pig serum was used as a source of complement only after previous absorption with the appropriate erythrocytes (1 ml packed erythrocytes per 5 ml serum for 30 minutes at 4°). Just before use, the serum was diluted five-fold with Eisen's B solution and 0·4 ml added to the cell mixture which was mixed and transferred to slide chambers from a Pasteur pipette by capillarity. The edges of the chambers were sealed by dipping them in molten wax. The chambers were incubated at 37° for 30 minutes. PFC were counted under a dissecting microscope (12·5 times magnification). The slide chamber was placed on a grid etched on a thick glass plate which was moved under the objective so the whole chamber was scanned. When there were only small numbers of plaques, they were ringed with a felt-tipped pen and examined under higher magnification to ensure that a lymphoid cell was present. The total number of PFC was expressed as the number of PFC/10^8 nucleated spleen cells. The appearance of one PFC in all the filled chambers used in a typical experiment represents a level of 5 PFC/10^8 spleen cells. It is possible to increase the sensitivity of the method by using a larger volume of spleen cell suspension and more chambers. This revealed that the level of PFC in non-immunized 6-day-old hamsters was less than 2 PFC/10^8 spleen cells. However, the use of three litters in order to perform one such determination was not considered justifiable for routine use.

Nordin, Cosenza and Hopkins (1969) have reported that IgM PFC were inhibited by concanavalin A in a localized-haemolysis-in-gel technique. The optimal conditions for inhibition of PFC (only 80 per cent inhibition) in our technique were obtained by preincubating the spleen cells with concanavalin A solution (0·03–0·05 mg/ml final concentration) at 20° for 15–20 minutes.

RESULTS

The ontogeny of the splenic PFC response with sheep erythrocytes as antigen is shown in Fig. 1. The number of 'natural' PFC in control hamsters rose rapidly after birth to adult level, in some cases as early as 9–10 days of age. Immunization of hamsters during the

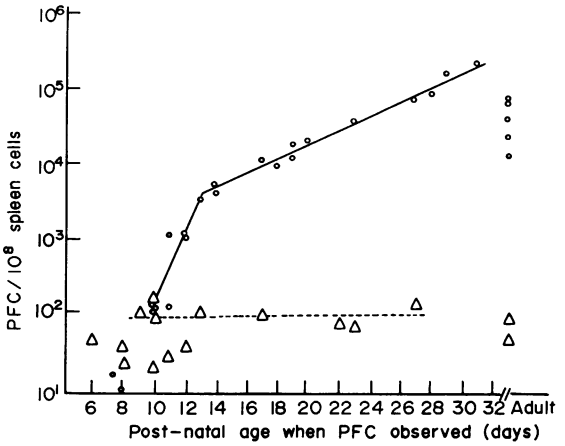

FIG. 1. Ontogeny of haemolytic plaque-forming cells in the spleen of hamsters antigenically stimulated with sheep erythrocytes, △, normal; ●, immunized. Each point represents one litter except in the case of adults when individual spleens were assayed.

first 6 days of post-natal life did not increase the level of splenic PFC, but by 11 days the immune PFC response was significantly greater than the level of 'natural' PFC. There was a rapid increase in numbers of PFC elicited by antigenic stimulation with age until 13 days and then a slower rate of increase up to at least 31 days. Immune PFC of 3-month-old adult hamster spleens were lower than those of younger animals.

This onset of ability to actively produce antibody was considerably later in development than the time predicted from the age-equivalence theory (Solomon, 1970, 1971a). Our recent observations in similar experiments with rats suggested that there may be suppressive delay in the onset of the response due to traces of anti-sheep antibody transmitted from the mother (Solomon, Riddell and Whyte, 1972). Certainly, when mouse erythrocytes were used as antigen a much clearer onset of PFC response could be observed (Fig. 2). This was partly due to the absence of any PFC in spleens of non-immunized

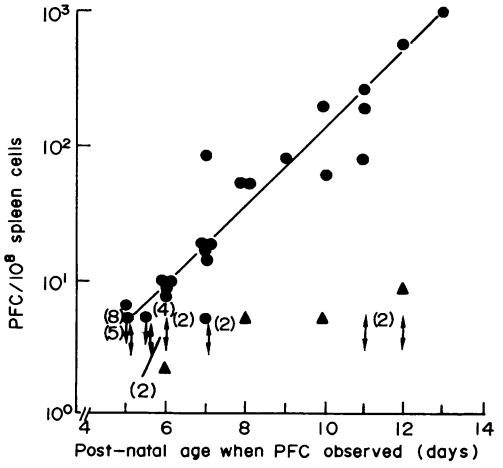

FIG. 2. Ontogeny of haemolytic plaque-forming cells in the spleen of hamsters antigenically stimulated with mouse erythrocytes, △, normal; ●, immunized. Each point represents one litter.

animals (except in one litter at 10 days of age). This increased the sensitivity of the method, as small numbers of plaques could be regarded as a significant increase above control levels. A two-fold increase in splenic PFC was observed 4 days after immunization of 2-day-old hamsters (Fig. 2). There was an exponential increase in numbers of PFC in spleens of immunized hamsters for at least 13 days after birth.

Mouse erythrocytes do not appear to be as immunogenic as sheep erythrocytes in hamsters at 14 days of age (cf. Fig. 1); this phenomenon has also been observed in rats (Solomon, Riddell and Whyte, 1972).

DISCUSSION

The onset of ability to produce haemolytic PFC in response to antigenic stimulation with sheep erythrocytes was originally claimed to be at 10 days after birth (Solomon, 1971b), but further determinations on spleens of non-immunized hamsters revealed an unexpectedly high level of 'natural' PFC at this age. However, our present results indicate a definite increase in immune PFC above the 'natural' level by 11 days after birth. Friedman and Goldner (1970) have recently detected 665 anti-sheep PFC in spleens of immunized hamsters at 8–10 days of age, although they gave no values for non-immunized hamsters. The very rapid appearance of relatively low levels of anti-sheep PFC (about 10 PFC/10^8 spleen cells) in apparently non-immunized hamsters during the first 10 days after birth suggests that the immunogenic stimulus due to common antigenic determinants in the diet may be stronger than previously supposed. This is emphasized by the failure of manual immunization via the peritoneal cavity to increase this small number of PFC.

The maturation of the PFC response to sheep erythrocytes follows a pattern of rapid increase from control levels at 10 days after birth to 16 days and then a slower exponential rise until at least 31 days when the response is somewhat greater than found for 3-month-old adults. Friedman and Goldner (1970) also observed a maturation of the PFC response up to adulthood. This maturation of the PFC response may be associated with increasing size of the thymus (relative to body weight) during the first 6 weeks of post-natal life which may result in increasing size of the 'helper' T cell pool. However, maturation (in the slower secondary phase) may be due to the additive effects of an increasing store of memory cells specifically committed by natural immunization by antigenic determinants common to sheep erythrocytes in the diet as well as possible sporadic infections.

When mouse erythrocytes were used as antigen, no PFC were detected in non-immunized hamsters during the first 10 days after birth and immune PFC could be detected only 6 days after birth. While this is the earliest reported active production of humoral antibody in the hamster, it is 3 days later than the onset predicted from the age-equivalence theory. Nevertheless, the hamster can make haemolytic antibody sooner than any other species when the chronological time-interval (9·5 days) between the first appearance of small lymphocytes in the thymus and antibody production is considered.

It has been suggested that some lymphoid cells may secrete less haemolytic antibody than others (resulting in plaques of different size) particularly during the early stages of ontogenetic development (Solomon, 1968). A very diffuse type of plaque has been observed by Jehn and Tannenberg (1970) in the early stages of a PFC response of adult mice; we have never observed such diffuse plaques in our experiments although there was the usual mixture of large and small plaques also typical of the adult response when sheep erythrocytes were used.

Evidence for the onset of immunocompetence in the hamster is fairly scant. The susceptibility of newborn hamsters to certain viral infections is well known. For example, resistance to an avirulent strain of mumps virus appeared as early as 7 days after birth and haemagglutinin-inhibiting antibody was detected 7 days later (Overman and Kilham, 1953). Plasma cells appeared 9 days after birth following neonatal infection with influenza virus, although no humoral antibody could be detected for another 11 days (Young, Ward, Hartshorn and Block, 1963). There is also evidence that 6–7-day-old hamsters begin to exhibit resistance to induction of tolerance with allogeneic lymphoid cells (Billingham, Sawchuck and Silvers, 1960). Our present results fall in line with the above results indicating an onset of immunocompetence in the hamster at 6–7 days after birth.

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