Changing Incidence of Positive Direct Coombs Test in Inbred NZB/BL Mice

R. D. BARNES, MAUREEN TUFFREY, and JEAN HOLLIDAY

From Department of Haematology, Institute of Child Health, University of London, 30 Guilford Street, London W.C.1

The inbred NZB/BL mouse has been shown by Bielschowsky, Helyer, and Howie (1959) to develop auto-immune haemolytic anaemia spontaneously, and this disorder has been considered to be genetically determined (Holmes and Burnet, 1966). Burnet and Holmes (1962) described a characteristic thymic change in this 'auto-immune' strain of mice; and certain renal lesions have been noted (Holmes and Burnet, 1963). The haemolytic anaemia in these mice has been associated with the presence of at least two different red cell auto-antibodies (Barnes and Tuffrey, 1966a). Both a positive direct Coombs test (DCT) antibody specific to mouse (Holmes and Burnet, 1963) and also an antibody which cross-reacts with mouse and human red cells (AHR) (Holborow, Barnes, and Tuffrey, 1965) have been described. Other auto-antibodies have been noted and include the LE cell factor (Helyer and Howie, 1961) and antinuclear factor (ANF) (Norins and Holmes, 1964; Holborow *et al.*, 1965). Like other workers (Holmes and Burnet, 1963; Norins and Holmes, 1964; East, Sousa, and Parrott, 1965; Miyasato, Marraligod, and Pollak, 1967), we have described the different age and sex incidence of certain of these spontaneously developing auto-antibodies since the original description of red cell auto-antibodies in the NZB/BL mouse by Bielschowsky *et al.* in 1959.

Using adoptive immunization techniques, we have shown the direct effect of the two red cell auto-antibodies in increasing *in vivo* red cell breakdown in the anaemic NZB/BL (Barnes and Tuffrey, 1966a, b). However, we have been unable to suggest any relation between the occurrence of ANF and disease in the NZB/BL (Holborow *et al.*, 1965). The finding of a high incidence of ANF in several 'normal' mouse strains (Barnes and Tuffrey, 1967) and our inability to relate the surprisingly high incidence of ANF in the NZB $\times$ CFW cross to any

definite auto-immune disorder, might cast some doubt on the auto-immune significance of ANF (Barnes, Tuffrey, and Berry, 1968).

In discussing a possible genetic mechanism which could account for the differing incidence in the auto-immune features of the disease process in the NZB/BL mouse, Holmes and Burnet (1966) suggested that such a mechanism would necessarily be complex. In an attempt to determine the genetic mechanism involved, we (Barnes *et al.*, 1968) and others (Helyer and Howie, 1961; Norins and Holmes, 1964; Holmes and Burnet, 1964; Burnet, 1965; Burnet and Holmes, 1965; Howie and Helyer, 1965; Holmes and Burnet, 1966) have performed hybridization experiments with other strains of mice, studying the comparative incidence of auto-immune features in the resultant crosses. Our findings confirm the complexity of any possible causative genetic mechanism which could account for the development of all the apparently unrelated and independent features of the 'auto-immune' NZB/BL mouse. In this study, on the other hand, we have examined the incidence of one particular antibody (DCT) in the inbred NZB/BL, to see if further inbreeding and the course of time caused any change in the age/sex incidence of this antibody.

Material and Methods

The NZB/BL mice were originally obtained in England through the Laboratory Animal Breeding Centre, Carshalton (L.A.C.). Animals from this in-bred strain, originally described in New Zealand by Bielschowsky *et al.* in 1959, were received in England at generation F. 53. Our animals, obtained after the mating of these original imported mice, were conventionally housed and bred at Taplow from March 1963 until January 1966, and during 1963-65 the incidence of spontaneously occurring auto-antibodies was determined (Holborow *et al.*, 1965). During this time the strain (referred to hereafter as the NZB/BL/A) was continuously inbred and the genetic integrity of the colony was confirmed by intra-strain skin grafting. In January 1966 the breeding nucleus of these animals at generation

F.61 was moved to its present conventional animal housing at the Institute of Child Health. Strict inbreeding has continued and this subline is now at generation F.66 (referred to hereafter as NZB/BL/T). A further nucleus of breeders was introduced in March 1966 from the L.A.C., Carshalton, at generation F.60. These animals were direct inbred descendants of the original NZB/BL stock imported from New Zealand and, having been maintained inbred, are currently at generation F.62 (referred to hereafter as NZB/BL/X). A third subline (referred to hereafter as NZB/BL/C) was derived from the mating of some mice of the same generation (F.60) but not brothers and sisters. These mice also originated from the L.A.C., Carshalton, and were obtained for 'traffic light' breeding (Lane-Petter, 1961), and after one mating were subsequently brother-sister mated. We have ensured the separate breeding of these three sublines, though housed together in conventional animal housing. Inter- and intra-subline skin grafting has been performed and no rejection has been noted up to 139 days after grafting.

Results and Discussion

The original age and sex incidence of the positive DCT in our conventionally housed NZB/BL colony was determined upon a cumulative basis. This present report represents more than 900 similarly derived results from animals not being utilized for separate experimental purposes.

Current results (NZB/BL/T, C and X), in comparison with our original findings (NZB/BL/A), show a delay in onset and a reduced incidence at all times of the positive DCT. These features are apparently not related to sex as the delay in onset and the reduction in incidence are observed in both males and females (Fig. 1 and 2). Analysis of the results in each of the three sublines showed a very similar pattern (Fig. 3).

These results might be very interesting genetically, suggesting that a process of 'selection' has occurred. Genetic selection in a highly inbred strain appears to be a contradiction in terms, but there is good evidence that such inbred strains retain genetic variability—perhaps as a result of heterozygote advantage (Wallace, 1965), so that selection is possible. Primary selection features could account for the current delay in the initial onset of the positive DCT. Particular notice is now being taken of the number of animals in each litter and their survival, and also the age and antibody state of the parents.

The reduction in positive DCT reactions currently detected at any one time might be due to one of several factors. This over-all reduction in incidence might represent the continuation of the

initial delay in onset throughout the period of investigation. On the other hand, the auto-immune haemolytic anaemia itself affects survival and will naturally influence selectively the proportion of DCT-positive animals available at any one time for investigation. However, the effect of death in selectively removing DCT-positive animals would be expected to influence the original results, as well as our current ones. Nevertheless, it is a possibility that the development of the DCT-positive reaction now renders the animals relatively more susceptible to death than it did originally, and that this might be due to an environmental factor. It may be argued that such an environmental factor might reduce the proportion of DCT-positive animals generally available for testing in the colony. It is difficult, however, to envisage an environmental factor which could cause the delay in the onset of a presumed genetically-determined positive DCT, and we must still consider this feature to be due possibly to a process of genetic selection.

Meanwhile, it is very important to record these preliminary results now, for several reasons. Hybridization experiments have frequently been performed to investigate the genetic features of the disease state by noting the incidence of the various auto-immune parameters (including the positive DCT) in the resultant cross (Helyer and Howie, 1961; Norins and Holmes, 1964; Holmes and Burnet, 1964; Burnet, 1965; Burnet and Holmes, 1965; Howie and Helyer, 1965; Holmes and Burnet, 1966). We wish to stress that if the positive DCT is the parameter, then the comparison between the results in the NZB/BL derived cross and the parent strain should be made at the same time and generation, and in the same environment, to achieve real significance. It is also important that results of the DCT obtained in different laboratories should not be directly compared without a certain degree of caution. Although our original over-all incidence of the positive DCT in the NZB/BL was similar perhaps in many respects to the pattern of results described by Holmes and Burnet in 1963, the current figures are now very different. Meanwhile, it is worth while noting that other workers have reported a differing over-all incidence of the Coombs test (East *et al.*, 1965; Miyasato *et al.*, 1967; de Vries and Hijmans, 1967); this is the first time, however, that a changing pattern has been noted within the same colony.

We are currently investigating whether there is a similar alteration in the incidence of the other spontaneously occurring red cell auto-antibody in the NZB/BL, the AHR (Holborow *et al.*, 1965).

FIG. 1. Incidence of positive direct Coombs test in male NZB/BL mice.

ANF is less likely to be genetically interesting, as in the NZB/BL and certain 'normal' mouse strains it appears to be directly influenced by environmental factors (Barnes and Tuffrey, 1967). Finally, the possibility of breeding NZB/BL mice with or

without the disease by 'selection', must not be underestimated. This would allow the true primary investigation of the disease state in the NZB/BL by providing animals at will, with or without the disease process.

FIG. 2. Incidence of positive direct Coombs test in female NZB/BL mice.

FIG. 3. Comparative incidence of positive direct Coombs test in 3 NZB/BL sublines.

Summary

The 'auto-immune' haemolytic anaemic NZB/BL mouse has been characteristically associated with the presence of a positive direct Coombs test (DCT). The age and sex incidence of the positive DCT in our colony of such mice has now been noted to be different from our original observations. The current delay in onset and the reduced number of DCT reactors in our colony, after a period of further inbreeding, might suggest a process of genetic selection, though other factors have yet to be excluded.

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