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Blood changes in experimental arthritis in two types of genetically different rats

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Summary

1. Rats genetically resistant to dextran and other agents producing the anaphylactoid reaction (NR rats), have a higher polymorph count than do rats which react to these agents (R rats).

2. NR rats do not develop polyarthritis when a hind paw is injected intradermally with Freund's adjuvant.

3. The polyarthritis produced by an intravenous injection of *Mycoplasma arthritidis* culture develops more slowly in NR rats than in R rats.

4. It is not clear whether the higher polymorph count in NR rats is a main factor in determining their resistance to adjuvant-induced arthritis.

Introduction

Whilst testing the sensitivity to clinical dextran of Wistar rats from different colonies, Harris & West (1963) found that more than one-fifth of the animals from one colony failed to respond with gross oedema of the extremities to the first dose and to all subsequent doses of dextran. By selective breeding experiments, Harris, Kalmus & West (1963) traced the failure to an inherited autosomal recessive character and a pure line of rats resistant to dextran (non-reactors or NR) was established. Later, Starr & West (1970) showed that NR rats form and release less histamine, 5-hydroxytryptamine and bradykinin in different shock states than do rats sensitive to dextran (reactors or R). Recently, Freeman & West (1972) reported that NR rats did not develop adjuvant-induced arthritis under conditions which resulted in extensive disseminated inflammatory lesions of joints and skin in R rats. As this disease resembles human rheumatoid arthritis in many respects, changes in blood parameters associated with the inflammation in R rats have been compared with those occurring in NR rats under the same treatment. In addition, the course of experimental *Mycoplasma* arthritis in both types of rat was followed as this model of inflammation is also of potential clinical interest (Eisen & Loveday, 1973).

Methods

Source of rats. Male Wistar rats of 150–210 g were used. The NR rats were obtained from the colony maintained at the North East London Polytechnic and the R rats came from A. Tuck and Son, Rayleigh, Essex. All animals had free access to food (diet 41B) and drinking water.

Blood techniques. Collection of blood at 9 h 00 min–10 h 00 min, and determinations of erythrocyte sedimentation rates (ESR), blood cell counts, total serum complement (CH50), complement component C3 and plasma lysozyme were carried out as described by Eisen & Loveday (1973).

Adjuvant-induced arthritis. Complete Freund's adjuvant (heat-killed *Mycobacterium tuberculosis* 0·25 mg in 0·05 ml liquid paraffin) was injected intradermally into the right hind paw of each rat. At intervals over 28 days, the developing polyarthritis was evaluated on an arbitrary scale from 0 to 3 units for each of the four limbs, two ears and tail: the maximum score per rat was therefore 21. In addition, the percentage increase in diameter of the injected paw was measured with a micrometer screw gauge. At different times after the injection of adjuvant, the response to dextran (mol. wt. 110,000) 180 mg kg^{-1}, i.p. was tested.

Mycoplasma arthritis. Rats were lightly anaesthetized with ether and 5×10^6 *Mycoplasma arthritidis* organisms in 0·5 ml of culture were injected into the tail vein of each. At intervals over 28 days, the arthritis was evaluated on an arbitrary scale from 0 to 6 units for each of the 4 limbs; an extra unit was added for each limb involved. The maximum score per rat was therefore 28.

Results

Blood parameters in untreated rats. The ESR, plasma lysozyme levels, total white blood cell (w.b.c.) counts, differential counts and complement levels in 25 R and 25 NR rats are shown in Table 1. Values in 90 R rats determined over the years 1971 and 1972 are included for comparison with the 25 R rats used in the present study. The most striking difference is the polymorph count in NR rats which is 2–3 times higher than in R rats. This usually, but not always, results

TABLE 1. *Blood parameters in untreated reactor (R) and non-reactor (NR) Wistar rats*

Parameter	Reactor (R) rats 1971 and 1972 (90 rats)	Reactor (R) rats Present study (25 rats)	Non-reactor (NR) rats Present study (25 rats)
ESR (mm/10 cm)	2·54±0·29	2·27±0·24	1·71±0·16
Total w.b.c. count	7,548±162	8,980±534*	13,916±744*
Polymorph count	1,525±98	1,274±104†	4,394±328†
Lymphocyte count	6,764±673	8,644±225	9,294±652
Monocyte count	179±24	160±18	176±24
Plasma lysozyme (Shugar units/ml)	152±17	160±17†	276±18†
Total complement (CH50 units/ml)	47·84±3·84	45·41±3·22	44·56±3·41
Serum C_3	89·30±2·00	91·00±2·03	95·97±1·92

Means±S.E.M. are given. Significant differences between R and NR rats in present study. *=$P<0·01$; †=$P<0·001$.

in a higher total w.b.c. count in NR rats. Plasma lysozyme levels are also significantly higher in NR rats. In 10 rats of each type, further estimations showed that the mean eosinophil counts (±S.E. mean) were 100±31 per mm^3 for R rats and 834±169 per mm^3 for NR rats, corresponding to 1·3% and 5·6% of the total w.b.c. counts, respectively; this difference is significant ($P<0·01$).

Adjuvant-induced arthritis. In preliminary studies in 15 R and 15 NR rats (Freeman & West, 1972), the primary response after Freund's adjuvant was found in all rats during the first 11 days, whereas the secondary disseminated lesions

of joints and skin occurred only in R rats and reached a peak about 22 days after injection (Figure 1A). Measurements of the diameter of the injected paw showed that the primary response in NR rats was less intense and the secondary response was absent (Figure 1B).

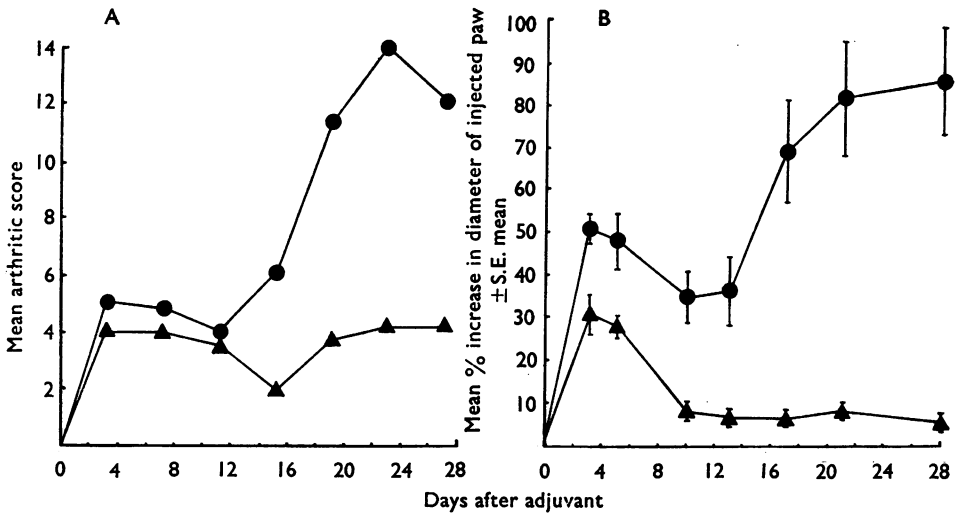

FIG. 1. Adjuvant induces the secondary polyarthritis in 15 R rats (●), but not in 15 NR rats (▲).

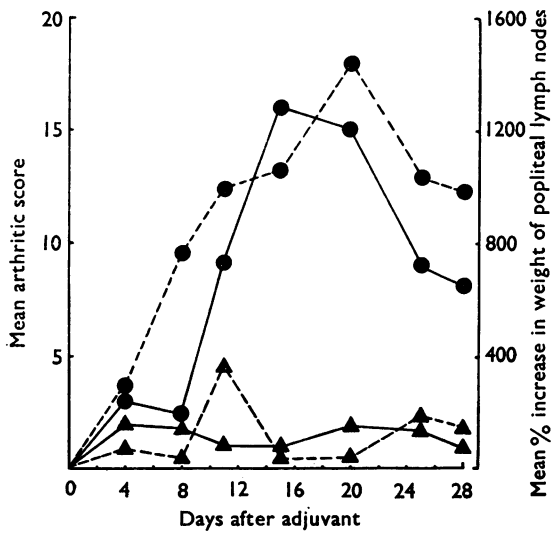

FIG. 2. Percentage increase in weight (- - - -) of popliteal lymph nodes of limb injected with adjuvant, follows total arthritic scores (——) in R rats (●) and NR rats (▲).

The percentage increase in weight of the popliteal lymph nodes draining the injected paw was also much greater in R rats (Figure 2).

Blood changes after Freund's adjuvant were analysed in a further experiment with 6 R and 6 NR rats. The ESR increased during the primary reaction in both types of rat but thereafter the increase in R animals correlated well with the intensity of the secondary symptoms of the disease (Figure 3A). The initial

total w.b.c. counts in these R and NR rats were similar, but from day 14 onwards the increase was greater in R rats (Figure 3B). The most striking changes, however, were found in the polymorph count; in R rats, this count increased sharply from about 2,000 per mm^3 to nearly 10,000 per mm^3 between days 7 and 14 and then remained high, whereas the count in NR rats stayed steady at about 5,000 per mm^3 throughout the 28 days of observation (Figure 3C). Plasma lysozyme levels closely followed the course of the disease in R rats, but little or no change occurred in NR rats. Lymphocyte counts showed no consistent changes during the disease, except for a transient rise at day 3, which was more pronounced in R rats. The initial complement values in these R and NR rats were low. Over the 28 days, total haemolytic complement (CH50) increased only in R rats (Figure 4). The third component of complement (C3) was raised in R and NR rats at day 3 (the primary reaction) but thereafter higher values were found only in R rats (Figure 4).

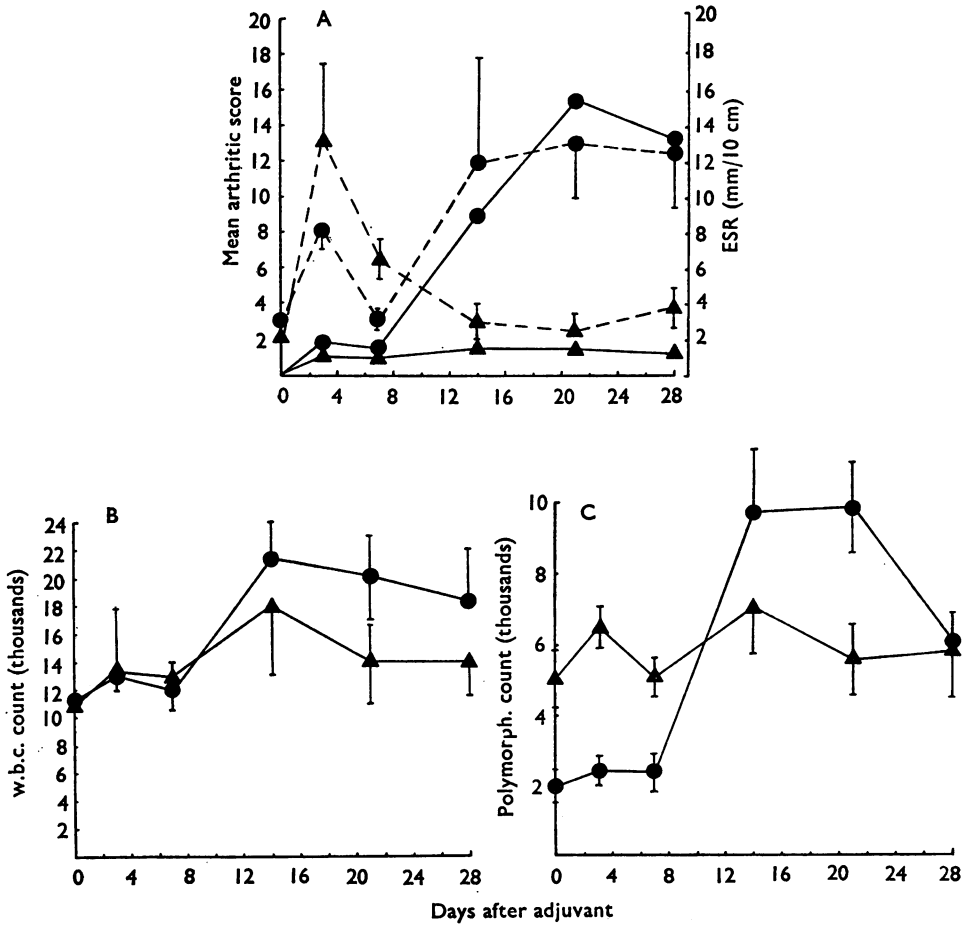

FIG. 3. During the adjuvant-induced arthritis (A, ——) in 6 R rats (●), the erythrocyte sedimentation rate (ESR) (A, – – – –), total white blood cell (w.b.c.) count (B) and polymorph count (C) were increased and followed changes in arthritic score. In 6 NR rats (▲), ESR showed a primary response, whilst w.b.c. and polymorph counts did not increase consistently over 28 days.

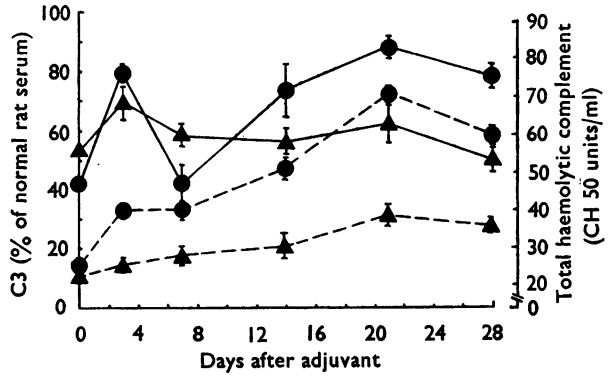

FIG. 4. During the adjuvant-induced arthritis, total haemolytic complement (CH50; ----) and component C3 (——) showed a primary and secondary increase in 6 R rats (●). In 6 NR rats (▲), CH50 showed only a slight increase over 28 days; C3 increased during the primary response.

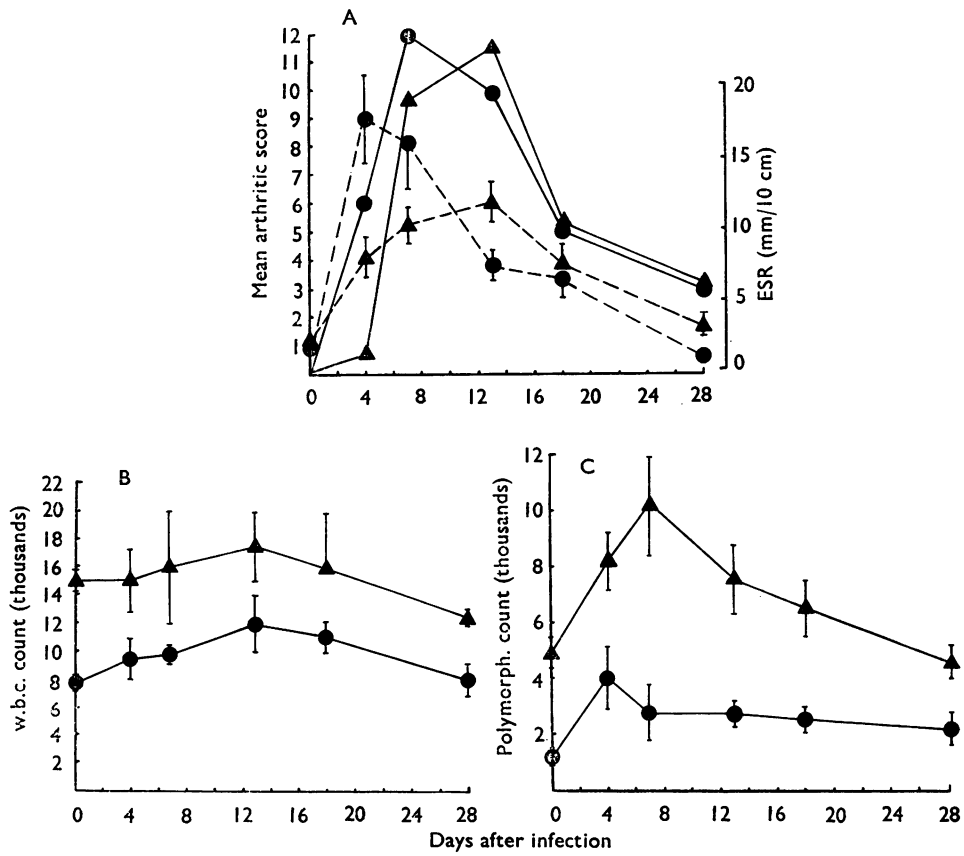

FIG. 5. During the polyarthritis (A, ——) produced by *Mycoplasma arthritidis* in 10 R rats (●), the erythrocyte sedimentation ratio (ESR) (A, ----), total white blood cell (w.b.c.) count (B), and polymorph count (C) were increased and followed changes in the arthritic score. In 10 NR rats (▲), arthritic score, ESR and polymorph count reached peak values later than in R rats.

Effect of higher doses of Mycobacterium tuberculosis. To determine whether the threshold to adjuvant was higher in NR rats than in R rats, responses to 10 times the original dose of mycobacteria in 0·05 ml of complete Freund's adjuvant were compared. Secondary arthritis was slightly more severe in 15 R rats than with the original dose but it still failed to develop in 15 NR rats.

Sensitivity to dextran. The observation that at the start of the treatment NR rats possess a higher polymorph count than do R rats raised the possibility that this may account for the resistance to dextran. The sensitivity of R rats to dextran was therefore tested during the secondary reaction when the polymorph count well exceeded that in untreated NR animals. However, these R rats responded well to dextran (with gross oedema of the snout, ears and paws) during the whole course of the 28 day period. NR rats failed on all occasions to give this anaphylactoid reaction.

Reaction to horse serum. Mycoplasma arthritidis is cultured in a medium containing 20% horse serum, and control experiments showed that this serum alone (0·1 ml) produced on intravenous injection into R rats a typical anaphylactoid reaction lasting 4–6 hours. No oedema of the snout, ears and paws occurred in any of 19 NR rats tested, thereby providing yet another difference in reactivity between the two types of rat.

Arthritis produced by Mycoplasma arthritidis. The onset of arthritis in 10 NR rats was delayed and its most severe phase developed later, but its intensity was similar to that in 10 R rats (Figure 5A). The recovery was also similar in both groups. Time courses of the disease were fairly closely reflected by changes in ESR (Figure 5A). The characteristic rise in total w.b.c. count (Eisen & Loveday, 1973) was roughly parallel in the two groups (Figure 5B). However, the peak counts in R rats (at day 13) were still below the initial values in NR rats. Polymorphs were mainly responsible for the increases in total w.b.c. counts (Figure 5C). Other blood changes were as previously reported (Eisen & Loveday, 1973) and were similar in R and NR rats. Lymphocyte counts first decreased and then rose. Plasma lysozyme levels remained slightly higher in NR rats. CH50 titres increased in R rats more than in NR rats.

Re-infection with Mycoplasma arthritidis. Six weeks after infection with *Mycoplasma arthritidis,* 10 R and 10 NR rats were re-infected. No arthritis developed in either group, suggesting that the immune response in NR rats is as effective as in R rats (Eisen & Loveday, 1973).

Discussion

The present results show that, in health, the total white blood cell count and in particular the polymorph count are significantly higher in rats which do not react to dextran (NR rats) than in rats which do (R rats). The eosinophil count of NR rats is also higher and forms a larger percentage of the total white blood cell count. These findings represent the only established major difference so far in physiological parameters between untreated R and NR rats. However, there is no evidence as yet that it is the more numerous polymorphs which endow the NR rats with the increased resistance to dextran and other shock procedures, as well as to complete Freund's adjuvant as shown in the present work. In fact, R rats still reacted to dextran during adjuvant-induced arthritis when their polymorph counts were even

higher than those in untreated NR rats. Furthermore, exchange transfusions of blood between R and NR rats did not change their reactivity to dextran (Ankier & West, unpublished work). The more numerous, and possibly more competent, polymorphs can therefore be, at most, one of several factors contributing to the increased resistance of NR rats. Alternatively, higher polymorph counts and greater resistance may be genetically linked without being causally related.

Polymorphs contain several factors which undoubtedly intensify inflammation and related processes. These include numerous lysosomal enzymes (Movat, 1971), some of them kinin-forming (Greenbaum, 1971); cationic mastocytolytic protein (Janoff, 1971); and factors chemotactic for lymphocytes (Ward, 1971). On the other hand, prevention or suppression of inflammation may be associated with lysosomal kinin-destroying carboxypeptidases (Greenbaum, 1971) and lysoszyme, or with the phagocytic activities of polymorphs which sequester provoking agents.

In their resistance to dextran and other shock procedures, NR rats resemble most wild rats (West, 1967). The reactions of the albino or piebald (partial albino) laboratory R rat may therefore be regarded as a dominant aberration from the normal genotype. Syndromes in which genetic predisposition to infectious and/or non-infectious diseases is linked with hypo-pigmentation and abnormal leucocytes are known in several species. Examples include the collagen-type disease of the blue-grey mink homozygous for the Aleutian gene, the susceptibility to infection of partial albino Hereford cattle, and the Chediak-Higashi syndrome in man characterized by hypo-pigmentation of eyes and skin, low red and white blood cell counts, typical cytoplasmic inclusions and peroxidase-positive lysosomes in polymorphs, and very low resistance to infections in spite of normal antibody formation. A basic point of difference, however, is that all these syndromes have a recessive mode of inheritance, whereas the reactivity of R rats is transmitted by a dominant gene (Harris *et al.*, 1963).

Infection with *Mycoplasma arthritidis* was equally effective in R and NR rats, although the onset of arthritis was delayed in NR rats. The further course of 28–30 days and the subsequent recovery were the same in R and NR rats. As previously reported, salicylates render this disease more severe (Eisen & Loveday, 1973). With other micro-organisms, clearer differences in the responses of R and NR rats to infections may be revealed.

The failure of Freund's adjuvant to produce arthritis in NR rats shows that they are more resistant not only to many acute noxious stimuli such as the injection of dextran but also to an immune reaction lasting several months and involving delayed hypersensitivity. This resistance of NR rats is of particular importance, since adjuvant arthritis is widely used in the study of human rheumatoid arthritis. This is mainly because the rat model is suppressed by numerous drugs used in the treatment of the human disease, as for example glucocorticoids, salicylates and other analgesics (Newbould, 1963; Winter & Nuss, 1966; Graeme, Fabry & Sigg, 1966), and immunosuppressive agents (Ward, Cloud, Krawitt & Jones, 1964). However, reports that gold salts improve adjuvant arthritis (Newbould, 1963; Walz, Di Martino & Misher, 1971) were not confirmed by Jessop & Currey (1968).

Finally, the widespread oedema produced in rats by horse serum i.v. provides yet another clear method of differentiating them from NR rats. This may be useful in experiments where administration of dextran is not desirable.

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