probably recognizes the E receptor has recently been obtained[12]. However, these antibodies cannot be used in gel diffusion or other methods involving precipitation, such as electroimmunodiffusion. The identification of R_s as a possible immunoregulatory substance and the demonstration of increased serum levels of R_s in diseases associated with depression of cell-mediated immunity opens a new line of investigation in clinical immunology.

1 Supported by FINEP, CNPq, CAPES and FAPESP.

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0014-4754/83/030306-03$1.50 + 0.20/0

Natural killer cell activity in fawn-hooded rats[1]

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Summary. Fawn-hooded (FH) rats were shown to lack the genetically conditioned defect of natural killer (NK) activity hypothesized to be present by analogy with the Chediak-Higashi syndrome (CHS) in mice and human beings. In 4-h ^{51}Cr release assays, splenic NK cells from FH rats killed YAC-1, RL♂1 and G_1-TC tumor targets without deficiency based upon comparison with cells from BD-IV, BD-IX and NBR inbred rat strains. Progeny of BD × FH F_1 rats backcrossed to FH failed to reveal a correlation of reduced NK activity and dilute coat color. From these, and other data presented, it is concluded that despite similarities in coat color dilution and platelet storage pool deficiency, FH rats do not closely resemble CHS mice or human beings in having deficient NK activity and cannot be considered the rat homolog of the CHS.

Fawn-hooded (FH) rats are mutants which manifest a genetic condition consisting of a coat color dilution and a platelet storage pool deficiency[2,3]. In these regards they resemble animals and human beings with the Chediak-Higashi syndrome (CHS)[4,5]. In addition to the coat color dilution and platelet storage pool deficiency, CHS mice and human beings have a marked deficiency of natural killer (NK) cell activity[6–8]. NK cells are lymphocytes which without antibody or previous exposure can kill certain tumor and virus-infected cells[9]. CHS mice, because of their lack of NK cell activity, have been used extensively to elucidate the mechanisms of NK cell activity and have proven useful in the demonstration of the in vivo relevance of NK cell activity[10]. In spite of the similarities of the conditions in CHS mice and FH rats no information is available regarding the NK cell activity of FH rats. The purpose of this investigation was to determine the levels of NK cell activity in FH rats.

Materials and methods. Animals. 6–12-week-old pedigree BD-IX, BD-IV, NBR and FH rats were used in these studies[1]. BD-IX, BD-IV and NBR are completely inbred strains. The FH animals used in this study were highly but incompletely inbred animals. BD-IV × FH F_1 and BD-IX × FH F_1 rats were obtained from matings between BD females and FH males. F_1 females were backcrossed to FH males and the progeny were used in these studies.

Cell lines and culture conditions. Three tumor cell lines sensitive to NK cell mediated lysis were employed[1]: YAC-1, RL♂1 and G_1-TC. These tumor target cell lines were cultured in HEPES buffered RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (complete HRPMI) as previously described[11].

NK cell mediated tumor cell lysis. For the completely inbred strains, splenic effector cells were prepared from a pool of 2 rats whereas individual rats were the source of effector cells for FH and backcrossed rats. Rat nylon-wool non-adherent spleen cells were obtained as previously described for the mouse[11] except that red cell lysis was achieved using a 6 sec rather than a 4 sec exposure to sterile distilled water. Nylon wool fractionation was carried out after preincubation at 37 °C for 18 h to allow for increased expression of effector reactivity[12]. Assays for NK cell reactivity were performed as previously described[10] using ^{51}Cr prelabeled target cells for the 4 h and ^{125}iododeoxyuridine (^{125}IUDR) prelabeled target cells for the 16 h assay. The percent specific release of ^{51}Cr or ^{125}I was determined using the following formula:

$$\frac{\text{counts released from experimental well} - \text{spontaneous release}}{\text{counts released from SDS lysed well} - \text{spontaneous release}} \times 100$$

The in vitro augmentation of NK activity was attempted by incubation of non-fractionated spleen cells for 18 h at 37 °C in complete HRPMI medium containing 50 µg/ml poly I:C and 25 µg/ml DEAE-dextran.

An outbreak of *Mycoplasma pulmonis*-associated respiratory disease which occurred in the room housing all the rat strains, afforded an opportunity to examine natural in vivo augmentation of NK activity. The splenic NK activity of exposed but clinically healthy rats was assessed during the second week of the disease outbreak.

Additional studies. In a further effort to determine how closely FH rats resembled CHS in other cell systems, hair and polymorphonuclear leukocytes, which, in animals with CHS, contain enlarged melanin and azurophil granules respectively, were obtained from FH rats. Blood was collected, smears were prepared and stained with Wright's stain. The cytoplasm of the polymorphonuclear leukocytes was examined microscopically for the presence of granules. Hair was clipped from the fawn portions of FH rats, mounted in immersion oil, and the melanin granules were examined and compared with those in the black hair of BD-IV and NBR rats.

Results. In the 4 h NK assays, spleen cells from FH rats had NK cell activity at least as high as that found for the NBR and BD-IX rat strains (table 1). The BD-IX strain is reported to have moderate to high NK activity[13] while the activity for BD-IV or NBR rats has not been previously reported. BD-IV rat spleen cells had a comparatively low activity against the mouse tumor cell targets, YAC-1 and RL♂1, but killed the rat lymphoma target, G_1-TC at a rate equal to splenic effectors from BD-IX or FH rats. Splenic effectors from NBR rats did not kill RL♂1 or G_1-TC cells as well as effectors from the other rat strains (table 1). All the progeny of BD × FH F_1 rats backcrossed to the FH strain had high levels of NK cell mediated tumor cell lysis (table 1). The backcross rats of BD-IV predigree did not have the low splenic NK activity noted in the parent BD-IV strain (table 1). In the 16 h NK assays, spleen cells from the FH strain had lower NK activity than those from BD-IV or BD-IX rats (table 2). The rat strains differed in their in vitro augmentation by poly I:C (table 3). The NBR rat effector cells were augmented to the greatest degree, the BD-IX rat cells to a lesser degree, whereas cells from both the BD-IV and the FH strains failed to develop augmented activity under the conditions used (table 3). During the outbreak of mycoplasma-associated respiratory disease, exposed, clinically healthy BD-IV, BD-IX and FH rats all had elevated splenic NK activity (table 4) compared to the activity of these rat strains before and after this episode (table 1). No differences were found for splenic NK activity between male and female rats and any time (table 4 and other data not shown).

No cytoplasmic granules were detectable by light microscopy in polymorphonuclear leukocytes from FH rats. The melanin granules in the hair of the FH rats were not enlarged compared to those present in the black hair of BD-IV or NBR rats.

Discussion. Mice with CHS have a platelet storage pool deficiency, a coat color dilution, a lack of NK cell activity, and an altered urinary excretion of lysosomal enzymes. Three other inherited coat color dilutions of mice (pallid, reduced pigmentation, and pale ear) are associated with altered urinary lysosomal enzyme excretion and a reduced activity of NK cells[14]. In addition to CHS, other human genetic conditions have been shown to have coupled partial albinism and reduced NK cell activity[15]. Although FH rats

Table 1. NK activity of rat spleen cells against various NK sensitive target tumor cell lines in a 4 h ^{51}Cr release assay at an effector:target ratio of 50:1[a]

Rat strain	Percent specific NK cell mediated target cell lysis[c] Target tumor cell line: YAC-1	RL♂1	G_1-TC
Experiment 1			
BD-IV	12	16	40
BD-IX	30	28	32
Fawn-hooded	43	28	46
NBR	24	8	17
Experiment 2			
BD-IV	15	12	ND[e]
BD-IX	39	35	ND
Fawn-hooded	33	23	ND
BD-IV. Fawn backcross[b], black phenotype	37	25	ND
BD-IV. Fawn backcross, fawn phenotype	31	18	ND
BD-IX. Fawn backcross, black phenotype[d]	41	25	ND
BD-IX. Fawn backcross, fawn phenotype	46	28	ND

[a]Rat spleen cells incubated overnight at 37 °C, subsequently passed over nylon wool. [b]Result of mating BD-IV × fawn-hooded F_1 female to fawn-hooded male. [c]All assay points the average of quadruplicate samples. [d]Similar results were obtained with agouti phenotype rats and no differences were found between solid and hooded offspring. [e]ND, not determined.

Table 2. NK activity in rat spleen cells against the NK sensitive tumor target cell line, YAC-1 in a 16 h $^{125}IUDR$ release assay[a]

Rat strain	Percent specific NK cell mediated target cell lysis 100:1[c]	50:1	25:1	12:1
BD-IV	ND[b]	32	19	8
BD-IX	ND	40	28	16
Fawn-hooded	24	18	14	0

[a]The average of 3 separate experiments all with similar results. All assay points represent the mean of quadruplicate samples. [b]ND, not determined. [c]Effector:target.

Table 3. NK activity of rat spleen cells against the NK sensitive tumor target cell line, YAC-1, with and without in vitro incubation with poly I:C[a]

Rat strain and regimen	Percent specific NK cell mediated target cell lysis 50:1[b]	25:1	12:1
NBR without poly I:C incubation	22	13	7
NBR with poly I:C incubation	54	31	13
BD-IX without poly I:C incubation	32	21	12
BD-IX with poly I:C incubation	47	31	19
BD-IV without poly I:C incubation	15	7	4
BD-IV with poly I:C incubation	15	9	5
Fawn-hooded without poly I:C incubation	28	15	10
Fawn-hooded with poly I:C incubation	27	17	9

[a]Spleen cell effectors incubated overnight at 37 °C in complete medium or complete medium containing 50 µg/ml poly I:C and 25 µg/ml DEAE-dextran before nylon wool fractionation. A 4-h ^{51}Cr release assay was employed where all assay points represent the mean of quadruplicate samples. [b]Effector:target.

Table 4. Effect of mycoplasma exposure in vivo on the NK activity of rat spleen cells towards the tumor cell line, YAC-1[a]

Rat strain	Percent specific NK cell mediated lysis 50:1[c]	25:1	12:1
BD-IV, male	33	14	10
BD-IV, female	34	20	13
BD-IX, male	59	37	19
BD-IX, female	ND[b]	ND	ND
Fawn-hooded, male	57	42	30
Fawn-hooded, female	58	38	26

[a]Exposed, apparently healthy rats assayed in the 2nd week of disease outbreak. 8 BD-IV, 8 FH and 4 BD-IX rats were assayed with similar results, and representative sample data are presented. A 4-h ^{51}Cr release assay was employed with all assay points the average of quadruplicate samples. [b]ND, not determined. [c]Effector:target.

have an inherited coat color dilution and a platelet storage pool deficiency this study demonstrated that they do not have a reduced level of NK cell activity. Because the level of NK cell activity varies among different strains of rats, and because the genetic background and origin of the FH rat are unknown, it was possible that the apparently normal non-reduced level of NK cell activity detected in the FH rats was in actuality a level reduced from that which would have been present in the same rats had they lacked the FH gene. If this had been the case, however, the non-fawn phenotypes of the rats obtained by the F_1 backcrosses would have had significantly higher levels of NK cell activity than those of the fawn phenotype obtained from the same backcrosses. Because they did not (table 1), it can be concluded that the FH gene in rats does not result in reduced NK cell activity.

The lack of enlarged cytoplasmic granules in the polymorphonuclear leukocytes and the lack of enlarged melanin granules in the hair of FH rats indicate that FH rats also differ in these regards from CHS. Many characteristics of NK activity in the rat have been found to more closely parallel those of man that does the NK system of the mouse. These include augmentation with interferon inducers[16], non-lability of the short-term activity at 37 °C, target selectivity of NK subpopulations[18,19], isolation characteristics for peripheral NK cells[17] and age dependency for maximal NK cell activity[18,20]. Because of the similarities between rat and man, the value of the rat for experiments on the biological relevance of NK cell mediated tumor lysis is becoming increasingly evident, and it would be highly desireable to have available a genetically conditioned deficiency of NK cell activity in the rat as is available in the CHS mouse. Unfortunately, the major conclusion reached in this study is that the FH rat has no such defect. The data obtained in this investigation was for rat effectors after augmentation of NK cell activity by incubation at 37 °C overnight[21]. CHS mice respond slowly to NK augmentation in vivo but may eventually show peak activities similar to their phenotypically normal littermate controls[22]. We considered the possibility that the overnight incubation might have allowed defective NK cells to augment to normal levels. This possibility was rejected for the following reasons. When we measured the NK cell activity of directly harvested spleen cells, the relative levels of activity among the various rat strains were similar to those of preincubated effectors (data not shown). Because CHS mice show slower augmentation of NK cell activity following treatment with interferon inducers, we postulate that we would still have seen lower activity for similarly defective rat effectors, particularly since the augmentation of the rat NK cell activity depends on macrophages of the same strain and not exogenously added inducers[12]. BD-IV and FH rats responded poorly if at all to poly I:C augmentation in vitro (table 3). This may indicate that these strains differ in their constitution of splenic cells contributing to the interferon response. The same rat strains responded well to a natural in vivo augmentation stimulus, mycoplasma infection (table 4), indicating that these rats do have adequate overall interferon responses.

In general the BD-IV rat strain exhibited lower splenic NK cell activity than the other rat strains when examined in the 4 h assay (table 1, 3 and 4). The exception was activity directed against the rat lymphoma target, G_1-TC which BD-IV effectors killed with a high level of activity. Recently some degree of heterogeneity for rat NK cell surface antigen expression has been demonstrated[23] and it is possible that in the rat, as is the case for man[24-26], different NK subpopulations may recognize and kill target cells of different antigenic specificities. Perhaps the BD-IV rat strain has a relatively greater complement of an NK cell population recognizing G_1-TC target cells. Similar reasoning may explain the low NK cell activity shown by NBR splenic effectors toward RL♂1 and G_1-TC targets (table 1).

In the 16 h ^{125}IUDR release assay the FH rat effectors exhibited the lowest NK cell activity indicating that FH effectors may not be as hardy as BD effectors in long-term culture.

1 Acknowledgments. This work was supported in part by grant RR00515 from the National Institutes of Health and grant RD78 from the American Cancer Society. We thank Jennie Hu for excellent technical assistance. We thank Dr J. Mayo, Mr C. Reeder, Prof. M. Rajewsky, and Dr W.J. Dodds for providing the rat strains and Drs J.M. Durdik and C.W. Reynolds for providing the tumor cell lines.

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