Time Dependence of the Number of Histocompatibility Loci in Skin Graft Rejection of Mice[1]

Our knowledge of the histocompatibility (H) systems is greater for the mouse than for any other species, and several experiments have been made in these rodents in order to evaluate the number of H loci and their relative strength. The estimates[2-6] obtained with distinctive methodologies and different strains range between 13 and 29. Such values are generally considered as minimum estimates, and pertinent reasons for that and for diversity of results are reported by BAILEY and MOBRAATEN[6]. The distinction between strong and weak H antigens has evolved from their different capacity to promote allograft rejection[7-9] and, since the spectrum of graft survival times is probably continuous[10], the strength of such antigenicities can be thought of as asymptotically degrading.

The experiments reported here were undertaken to estimate the number of H loci at which male and female mice of DBA/2 and C57BL/6 inbred strains differ, and to evaluate their relative strength by the proportion of them operating at various intervals after grafting.

Materials and methods. Graft recipients (F_3) were obtained from the mating of the backcrosses (C57BL/6♀ × DBA/2♂) F_1♀ × DBA/2♂, and skin donors were DBA/2 mice. Assuming that H genes are codominant, the expected proportion of susceptible mice will be in our F_3 $(15/16)^n$, where n represents an ideal number of independently assorted and fully expressed loci able to cause rejection in 100% of animals. Transplants were performed between members of the same sex, aged 3–6 months, according to the technique of BILLINGHAM and MEDAWAR[11], by grafting about 0.5 cm² of DBA/2 ear skin on the back of F_3 mice. The grafts were inspected through 240 days and they were deemed to be rejected when an initial damage was present. The acceptance was expressed as percentage $100 \times (S/N)$, and standard errors were estimated by the formula: $\pm \sqrt{\frac{\frac{S}{N} \cdot \left(1 - \frac{S}{N}\right)}{N}}$,

S being the number of animals with surviving grafts and N the number of animals in the group. Regression lines ($Y = a + bX$) for the regression of the number of operating H loci (Y) on log time (X) and their confidence limits were calculated[12].

Results and discussion. The Table gives, separately for males and females, the number and percentage of acceptance and the corresponding number of operating H loci at various time intervals after grafting. In the Figure the number of operating H loci is plotted against the $\log_{10}$ of the time in days. At 240 days the estimated number of H loci at which strains DBA/2 and C57BL/6 differ was 28.5 in males and 44.1 in females. It would be meaningless to compare such values with those found by other authors, since they are quite certainly an understimate of the number of genes involved and depend on the strains and on the sensitivity of the method used[6]. We prefer, instead, to discuss some other aspects of our results.

Data show firstly that in both males and females there was a continuous spectrum of graft survival times ranging from 9–10 to more than 200 days. It is evident that the building of the immune responses elicited by different H antigens takes various times to reach an effective level. As a consequence, the number of operating H loci, i.e. those able to cause rejection, increases with time. Holding in due consideration that our values do not discriminate the possibility of linked or partially expressed loci, it results from the Figure that the relationship between the number of H loci and the log of the time can fit a linear function in both groups of animals, which means that the rate of increase of the number of active H loci is inversely proportional to time. The reasons for this particular distribution are not understood. The equations

[1] This investigation was supported by a grant of the Consiglio Nazionale delle Ricerche.

[2] G. D. SNELL, J. Genet. *49*, 87 (1948).
[3] A. D. BARNES and P. L. KROHN, Proc. R. Soc. London B *146*, 505 (1957).
[4] R. T. PREHN and J. M. MAIN, J. natn. Cancer Inst. *20*, 207 (1958).
[5] C. K. CHAI and M. S. M. CHIANG, Genetics *48*, 1153 (1963).
[6] D. W. BAILEY and L. E. MOBRAATEN, Transplantation *7*, 394 (1969).
[7] R. C. ELANDT-JOHNSON, Transplantation *6*, 481 (1968).
[8] C. F. MCKHANN, Transplantation *6*, 482 (1968).
[9] J. H. STIMPFLING, Transplantation *6*, 489 (1968).
[10] R. J. GRAFF, W. H. HILDEMANN and G. D. SNELL, Transplantation *4*, 425 (1966).
[11] R. E. BILLINGHAM and P. B. MEDAWAR, J. exp. Biol. *28*, 385 (1951).

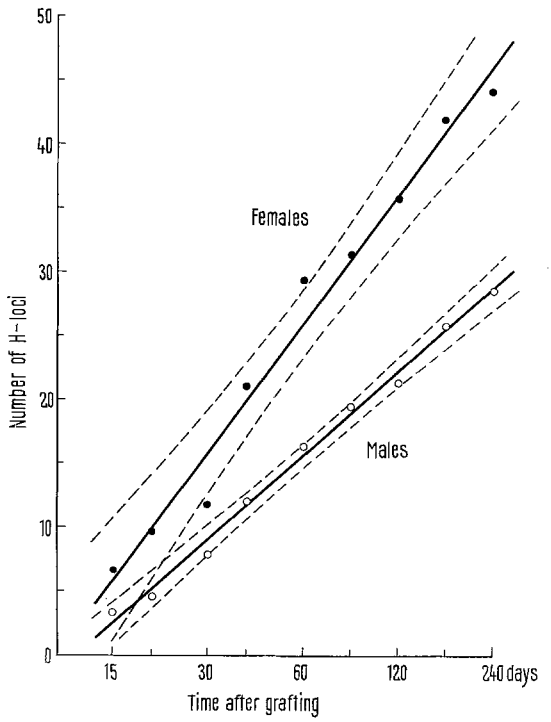
Distribution of survival of grafts from DBA/2 donors on male and female F_3 hybrids

Days after grafting	Control males (63)[a] No. recipients with surv. grafts	Acceptance (%) ± S.E.	No. operating H-loci[b] ± S.E.	Control females (120)[a] No. recipients with surv. grafts	Acceptance (%) ± S.E.	No. operating H-loci[b] ± S.E.
15	51	81.0 ± 4.9	3.3 (2.4–4.3)	78	65.0 ± 4.4	6.7 (5.7–7.8)
20	47	74.6 ± 5.5	4.5 (3.4–5.7)	64	53.3 ± 4.5	9.7 (8.5–11.1)
30	38	60.3 ± 6.2	7.8 (6.3–9.5)	56	46.7 ± 4.6	11.8 (10.3–13.4)
40	29	46.0 ± 6.3	12.0 (10.0–14.3)	31	25.8 ± 4.0	21.0 (18.8–23.6)
60	22	34.9 ± 6.0	16.3 (13.9–19.2)	18	15.0 ± 3.3	29.4 (26.3–33.2)
85	18	28.6 ± 5.7	19.4 (17.0–22.8)	16	13.3 ± 3.1	31.3 (28.0–35.4)
120	16	25.4 ± 5.5	21.2 (18.2–25.0)	12	10.0 ± 2.7	35.7 (32.0–40.6)
170	12	19.0 ± 4.9	25.7 (22.2–30.4)	8	6.7 ± 2.3	41.9 (37.3–48.4)
240	10	15.9 ± 4.6	28.5 (24.6–33.8)	7	5.8 ± 2.1	44.1 (39.3–51.1)

[a] Number of grafts judged technically successful on the 8th postoperative day. [b] The number (n) of operating H-loci was estimated by the formula $n = (\log S/N)/(\log 15/16)$, being S the number of compatible grafts observed in the sample of N grafts.

for the regression lines and the corresponding correlation coefficients were: $Y = -23.14 + 21.75\,X$ ($r = 0.997$) in males and $Y = -33.30 + 33.30\,X$ ($r = 0.989$) in females. In short, one clear conclusion emerges: the number of H loci at which two strains differ is not a fixed one but depends on the time elapsed between grafting and observation. Moreover, if the strength of H loci is measured in terms of survival time, our results confirm the presence of a continuous spectrum of H loci[10] whose strength is geometrically degrading.

In the second place, it is apparent from results that allograft rejection was slower in males than in females, in the same way for all the loci. The lines expressing the values of males show, in fact, a smaller slope than that of females, and the difference between the two corresponding regression coefficients is highly significant ($P < 0.001$). Our evidence of a greater immunological responsiveness of females agrees with the findings of other authors that females of a given strain of mice often reject skin allografts more rapidly than males[10, 13, 14], and are less susceptible to the growth of certain transplanted tumors[15, 16].



Relationship between the number of H loci and $\log_{10}$ of the time in days after grafting, in male and female F_3 hybrids grafted with DBA/2 ear skin. The broken lines represent the confidence limits of the regressions for 99/100.

Riassunto. I dati esposti indicano che l'incremento del numero dei loci dell'istocompatibilità ai quali differiscono i due ceppi di topi «inbred» DBA/2 e C57BL/6 è inversamente proporzionale al tempo trascorso dal trapianto, ed è maggiore nelle femmine.

G. Brambilla, M. Cavanna, S. Parodi and L. Baldini

Department of Pharmacology of Genova University, Viale Benedetto XV, I-16132 Genova (Italy), and Department of Pharmacology and Pharmacognosy of Trieste University, I-34127 Trieste (Italy), 4 March 1970.

[12] N. T. J. Bailey, in *Statistical Methods in Biology* (The English University Press Ltd., London 1968), pp. 24, 95.
[13] W. S. Lapp and J. Q. Bliss, Immunology *12*, 103 (1967).
[14] M. Galton, Transplantation *5*, 154 (1967).
[15] L. Gross, Proc. Soc. exp. Biol. Med. *49*, 67 (1942).
[16] G. D. Snell, J. natn. Cancer Inst. *20*, 787 (1958).

Cellular Antigens in Myxo- and Paramyxoviruses as Revealed by Immunodiffusion Methods

Antigens of cellular origin have been revealed in the envelope of myxo- and paramyxoviruses which appeared to be species-specific[1], group-specific[2] and heterogeneous[3]. Our attention was drawn to immunodiffusion methods which have been explored in the study of antigenic pattern of some viruses[4].

Materials and methods. Experiments were conducted with fowl plague virus (FPV, strain Weybridge), Newcastle disease virus (NDV, strains Tomilin and Herfordshire), and Sendai virus (strain 960) grown in chick embryos and purified in DEAE cellulose[5]. S and V antigens of FPV were obtained by the method of Hoyle[6]. Immune sera were obtained by quadruple intramuscular inoculation of purified virus preparations with one-week intervals. S and V immune sera were obtained by the method of Lief and Henle[7]. Cellular extracts (10%) in saline were used as cellular antigens, extracts of guinea-pig kidney and rabbit erythrocytes were used as Forssmann's antigen, human group A erythrocytes were used as group-specific antigen.

The following immunodiffusion methods were used: double diffusion[8], immunoelectrophoresis[9], and immunoosmophoresis[10].

Results and discussion. In double diffusion experiments both virus-specific and cellular antigens were revealed in purified virus preparations. Figure 1 shows the results of an experiment in which purified concentrated FPV (III, VIII) was subjected to interaction with FPV (1, 10, 11, 13) and NDV (23, 24) immune sera. It is seen that both sera interact with FPV antigen as well as anti-charioallantoic serum (18) and group B human serum (26), the latter specifying the presence of group A antigen in

[1] P. N. Kosyakov and Z. I. Rovnova, Vop. Virus. (Russian) *10*, 17 (1965).
[2] G. F. Springer and H. Tritel, Science *138*, 687 (1962).
[3] R. Drzeniek, M. S. Sober and R. Rott, Z. Naturforsch. *21b*, 254 (1966).
[4] H. W. I. Ragetti and M. Weintraub, Science *144*, 1023 (1964).
[5] G. K. S. Chepulis, A. F. Bocharov and V. M. Zhdanov, Vop. Virus. (Russian) *4*, 439 (1967).
[6] L. J. Hoyle, Hygeia *30*, 229 (1952).
[7] F. S. Lief and W. Henle, Virology *2*, 753 (1956).
[8] O. Ouchterlony, Ann. N.Y. Acad. Sci. *121*, 640 (1953).
[9] G. Grabar and C. A. Williams, Bioch. biophys. Acta *10*, 193 (1953).