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Parabiotic Anemia-Polycythemia.* (26363)

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Surgical parabiosis of genetically diverse animals frequently results in severe and persistent anemia of one partner and polycythemia of the other(1,2,3). This may be observed as early as the third day after junction. In the absence of genetic diversity this is rare, and, if present, only mild and of short duration. Anemia-polycythemia (AP) has been most commonly observed in instances of unilateral genetic diversity, *i.e.*, after parabiosis of a mouse of one inbred strain with its F_1 hybrid(4). AP has been interpreted generally as due to an immune response of the inbred mouse against the unrelated parent component of the hybrid. In this combination the hybrid becomes anemic and the inbred mouse polycythemic, a development probably brought about by a shift of red cell mass from the hybrid into the inbred mouse. If the AP were the result of an immune response to incompatible tissue, it could be used as an assay of this response. However, this assay should only be of aid in unilateral immune responses. Bilateral immune responses, both aiming at AP, should tend to cancel each other.

The data presented here were obtained from mice of 2 unrelated strains, parabiosed to teach other and to their common F_1 hybrids. They suggest that at least 2 separate factors operate to bring about AP, only one of them being immunologic in nature.

Material and Methods. Experimental animals were inbred mice from our colonies, of the C57BL/6 and BALB/c strains, and their reciprocal hybrids. Partners were of the same sex, with approximately equal numbers of male and female pairs. The technique of parabiosis has been described(3). Blood for microhematocrit determination was obtained from blood of the tail vein. The mice were bled on the 6th, 10th, 13th, and 17th days after parabiosis.

Observations. Parabiosis of *BALB/c mice with (C57BL/6 x BALB/c)F_1* hybrids resulted in AP on the 6th day in all pairs, invariably "favoring" (= creating polycythemia in) the BALB/c partner (Table 1). It increased in severity until the end of the 3rd week when death had occurred in most instances.

Parabiosis of *C57BL/6 mice with (C57BL/6 x BALB/c)F_1* hybrids did not result in similar AP on the 6th day. Instead, 18 of 25 pairs showed a moderate AP favoring the *hybrid* partner (mean: 15.7 S.D. $\pm$ 8.4), while the remaining 7 pairs showed mild AP favoring the C57BL/6 (mean: 9.4, S.D. $\pm$ 8.1). However, the trend reversed itself on successive bleeding days; results became uniform, all pairs showing AP favoring the C57BL/6 side, but significantly less severe than in the BALB/c-hybrid combination ($p<0.02$).

Parabiosis of *BALB/c with C57BL/6* mice

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TABLE I. Hematocrit Values of Parabiosed Incompatible Mice.

Day	No. of pairs	Mean hematocrit BALB/c	Mean hematocrit F₁	Mean hematocrit difference
(a) BALB/c – (BALB/c × C57BL/6)F_1				
6	13	65.0 ± 3.26*	38.8 ± 5.36*	26.2 ± 6.75*
10	17	74.8 ± 4.79	21.3 ± 6.94	53.5 ± 34.0
13	7	75.6 ± 5.11	18.7 ± 4.46	56.9 ± 6.41
17	9	73.9 ± 18.10	20.6 ± 17.10	53.3 ± 36.90
(b) C57BL/6 – (BALB/c × C57BL/6)F_1				
		C57BL/6	F_1	
6	25	45.4 ± 8.21*	54.0 ± 7.38*	-8.6† ± 14.09*
10	16	57.9 ± 14.80	29.9 ± 10.4	28.0 ± 22.30
13	14	58.7 ± 11.90	22.3 ± 8.62	36.4 ± 19.27
17	11	55.7 ± 11.3	23.0 ± 11.30	32.7 ± 22.05

Day	No. of pairs	Mean hematocrit BALB/c	Mean hematocrit C57BL/6	Mean hematocrit difference
(c) BALB/c – C57BL/6				
6	15	64.7 ± 3.32*	29.0 ± 9.78*	35.7 ± 12.80*
10	7	66.0 ± 10.40	29.7 ± 12.0	36.3 ± 23.84
13	6	61.7 ± 11.40	33.2 ± 11.0	28.5 ± 22.50
17	5	55.2 ± 9.69	37.0 ± 5.02	18.2 ± 15.50
(d) Normal hematocrit values of strains				
			No. of mice	
BALB/c			51	50.3 ± 2.89*
C57BL/6			8	51.8 ± 1.66
(BALB/c × C57BL/6)F_1			42	51.0 ± 1.70

* Stand. dev.

† Hybrid polycythemia.

resulted in AP on the 6th day in all pairs, which uniformly favored the BALB/c partner, and was significantly more severe than in the BALB/c-hybrid combination at that time ($p<0.02$). However, it failed to increase in severity, and on the 10th day was significantly below that seen in the BALB/c-hybrid combination ($p<0.02$). AP persisted at about the same level until the 13th day after which it decreased somewhat.

Discussion. The results indicate that factors other than, or in addition to, mutual immune responses (BALB/c vs. C57BL/6, and C57BL/6 vs. BALB/c) operate to bring about AP in parabiosis. 1) The BALB/c-C57BL/6 combination brought about more severe AP on the 6th day than either pure-hybrid combination. Mutual immune responses, competing with each other, would have had the opposite effect. The initially lesser degree, when one of the partners was a hybrid, suggests a genetic dilution effect that is usually not associated with genetically determined antigens concerned with tissue incompatibility. 2) The initial AP in the C57BL/6-hybrid combination favoring the hybrid, and followed by its reversal, is not readily explained by an immune response. Polycythemia has been observed in animals *exerting* an immune response against their partners, and hybrids do not react immunologically against their parent strains(3).

We wish to propose the following hypothesis: A *primary* AP may occur between incompatible parabiotic partners as soon as adequate vascular anastomoses have developed. It is unrelated to immune responses, conceivably related to differences in blood pressure between strains, and expresses itself in hybrids in a dilute fashion. It favors the BALB/c over the C57BL/6, their mutual F_1 hybrid occupying an intermediate position. An immune response may give rise to *secondary* AP which is superimposed on the primary, and overrides it. Fig. 1 to 3, in which hematocrit differences are plotted against time, are an attempt to explain the observations diagrammatically on the basis of these postulates. Points at the level of the abscissa indicate absence of a hematocrit difference;

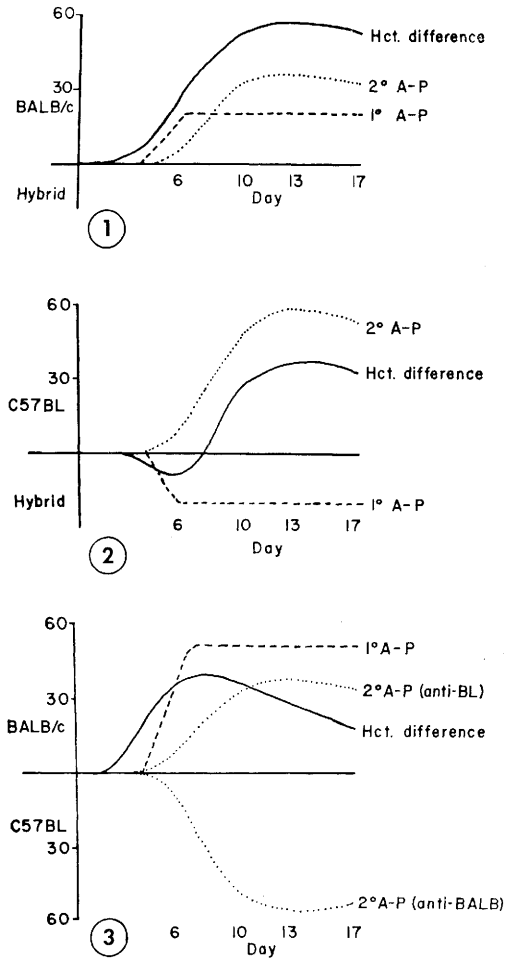

FIG. 1. Mean hematocrit data of BALB/c and (C57BL × BALB/c)F_1 mice joined in parabiosis (see text).

FIG. 2. Mean hematocrit data of C57BL/6 and (C57BL/6 × BALB/c)F_1 mice joined in parabiosis (see text).

FIG. 3. Mean hematocrit data of BALB/c and C57BL/6 mice joined in parabiosis (see text).

points above and below indicate a polycythemia of the partner shown at the left (e.g., in Fig. 1, hematocrit values of BALB/c mice are higher than those of their hybrids).

BALB/c-hybrid (*Fig. 1*): The primary AP (broken line) favors the BALB/c partner, but is of only moderate strength. The secondary AP (dotted line) also favors the BALB/c (since the hybrid can not react against the BALB/c); it begins to make itself felt already on the 6th day, but increases in severity thereafter. It is superimposed on the primary AP as a result of which both anemia and polycythemia are more severe than in the other two combinations.

C57BL/6-hybrid (*Fig. 2*): The primary AP favors the hybrid. The secondary AP favors the C57BL/6, partly cancels the effect of the primary AP on the 6th day, and overrides it on successive bleeding days. It never reaches the level of the AP in the BALB/c-hybrid combination because of the persistent opposing effect of the primary AP.

C57BL/6-BALB/c (*Fig. 3*): The primary AP favors the BALB/c, and is stronger than in the BALB/c-hybrid combination. The secondary responses pull both ways, and essentially cancel each other; in consequence, there is no further increase above the level of the 6th day. The decrease in AP during the 2nd week is probably the result of the readily demonstrable narrowing of vascular anastomoses between bilaterally incompatible partners(4).

Conclusions. Hematocrit data from incompatible mice joined in parabiosis suggest that a primary and a secondary anemia-polycythemia may develop. The secondary anemia-polycythemia appears related to an immune response, while the primary does not. The two events may counterbalance or intensify each other, depending on the genetic backgrounds of the mice. Because of this multiplicity of factors, the anemia-polycythemia of parabiosis appears unsuitable as an assay of the host response to incompatible tissue.

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