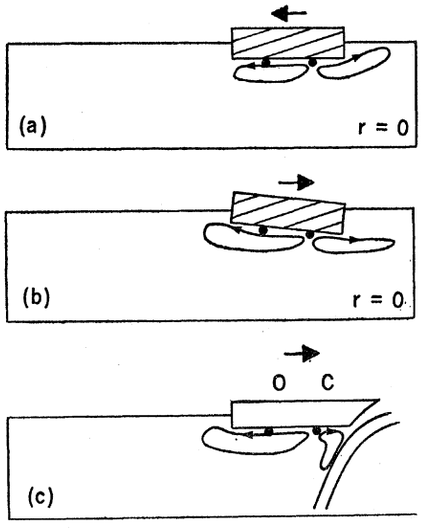

Fig. 2. Schematic diagrams of (a) the flow about a plane raft with the heater at the left inert, propelling the raft to the left; (b) the flow about a slightly inclined plane raft with the heater at the left inert, propelling the raft to the right; (c) the flow about a lithospheric raft with the oceanic heater inert; the raft is propelled to the right because of the downward deflection of the flow pattern at a convergent zone. Symbols are *O*, ocean; *C*, continent.

and ν the kinematic viscosity. The results are not inconsistent with the theoretical result that the velocity should vary as

$$R_1^{1/2}(1 - r)/(1 + r)^{1/2} = (R_1 - R_2)/(R_1 + R_2)^{1/2}$$

where R_1 and R_2 are the scaled heater strengths of the hotter and cooler elements, respectively. The theory has been developed for isothermal and free upper and lower surfaces, but we believe that the dependence on these variables should not change with a change in the boundary conditions; only the numerical coefficients in the solution should change.

The magnitudes of the velocities for the frame raft are of the order of centimeters per year for suitable values of the parameters. For a heat flux of 1 μcal/cm^2 sec for a continent of width 3000 km, and the values 600 km for h, 3×10^{-2} cm^2/sec for κ, 10^{21} cm^2/sec for ν, and 2×10^{-5} (°C)$^{-1}$ for α, we get velocities of the order of 2 cm/year if $r = 0$ and the heater separation b is set equal to h. Thus, it seems plausible that a lithospheric raft could be self-propelled with appropriate velocities because of the differences in heat production between its continental and oceanic parts.

Plane rafts were also constructed from sheets of hollow plastic, and the two heaters were symmetrically fixed on the lower surfaces. Motion was observed for this model for the case $r = 0$. If the raft were infinite in lateral extent, the symmetry for the case $r = 0$ would require that the raft be stable. Hence, we conclude that an elongated convection pattern, as earlier illustrated by markers, couples to the edge of the finite raft and thus provides an asymmetric drive on the raft (Fig. 2). The experimental results show that the velocity varies as $R^{1/2}$ for large R, as in the case of the frame raft, but falls to lower values at lower R; similar behavior has been observed by Whitehead (*3*) for a line heater suspended beneath a raft. Little dependence on r is found, except close to the singular case $r = 1$.

A lithospheric raft driven by asthenospheric heating under continents, as described here, would move toward the cooler regions, that is, the oceans. This would seem to contradict the behavior near the oceanic ridges. However, we were able to obtain reversal in the direction of motion consistently in experiments with plane rafts (with $r = 0$) inserted at a slightly nonlevel attitude, with the hot side depressed. We infer that the inclination of the raft depressed the circulation pattern near the hot end and allowed the long-range circulation to the cold edge to dominate. The geophysical implication is that the flows may be deflected and depressed by downthrust lithospheric slabs and that propulsion comes from the parts of the earth where the heat is most readily transported to regions near the surface (Fig. 2). In the earth, this is accomplished most effectively at the oceanic rises. There is an additional influence because the boundary conditions at oceanic rises are different from those described here.

This model has the virtue that relatively uniform velocities will be maintained as long as the conditions at oceanic rises remain relatively permanent. A literal result of the model is that plates without continental parts should be stable; two such plates are the Pacific and Nasca plates. These should have rather small relative motions, except as they may be driven through interaction with the other major plates of the world by virtue of closure on an almost spherical earth. Our model has not provided for this interaction.

L. KNOPOFF
K. A. POEHLS, R. C. SMITH
Department of Physics and Institute of Geophysics, University of California, Los Angeles 90024

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Genetic and Immunological Complexity of Major Histocompatibility Regions

Abstract. *There are genetic differences within the major histocompatibility complex of the mouse which lead to skin graft rejection but which cannot be detected serologically. When confronted with these differences on allogeneic cells, lymphocytes proliferate in vitro. In other cases, in vitro lymphocyte proliferation but no skin graft rejection is associated with loci that are linked to but genetically separable from the loci controlling the serologically defined antigens.*

There is a single major histocompatibility complex (MHC) in the genomes of mouse (*1*) and of man (*2*, *3*). Within that region in each species there are two loci approximately 0.5 recombinational units apart; alleles of these loci control serologically (antiserums) detectable antigens. We refer to these as serologically defined (SD) loci. In the mouse the SD loci are the H-2K and H-2D loci of the H-2 system; in man the LA and four loci of the HL-A system. Each locus is highly polymorphic with at least one antigen associated with each allele.

In addition there are genetic differences in the MHC which are at the very least difficult to detect serologically but which lead to a lymphocyte response in vitro. We refer to such differences as lymphocyte defined (LD) differences. We use the abbreviations

MHC, SD, and LD to simplify discussion of the findings without biasing the biological conclusions.

A simplified genetic map of the mouse MHC is given in Fig. 1a. The H-2K locus is proximal to the centromere and is thus written to the left of H-2D. Between these loci is the Ir-1 (immune response) locus (*4*), perhaps other Ir loci that control the response to a variety of antigens, and the Ss-Slp locus. The Ss locus controls the quantitative level of a serum protein; the Slp locus (which may be the same as the Ss locus) controls the presence of a serum antigen (*5*). This antigen is expressed only in males, and then only if the Slp^a gene is present.

Differences for the MHC between donor and recipient lead to graft rejection, which is usually rapid and relatively difficult to overcome with immunosuppressive agents. In addition to the MHC there are many minor loci segregating independently of the MHC which, if different in two individuals, can lead to graft rejection. Our present concept is that a recipient recognizes foreign (nonself) SD loci antigens on the grafted tissue, which leads to an immune response resulting in graft rejection. We limit our discussion here to the MHC.

The phenotypic expression of the MHC can be assayed in vitro both by the use of antiserums directed at the SD loci antigens and by the mixed leukocyte culture (MLC) test. Antiserums can be used to detect antigens on the surface of cells by several test procedures—the most common being cytotoxicity and agglutination. If a number of different antiserums are used, antigens of the SD loci, which characterize the phenotype of any individual, can be determined. Pairing of donor and recipient for transplantation is accomplished by reducing to a minimum the number of antigens by which the donor differs from the recipient. In the MLC test, responding lymphocytes of a recipient (for example, A) recognize "foreignness" on the stimulating lymphocytes of the donor (for example, B). Stimulating cells in one-way MLC tests are "inactivated" with mitomycin C (subscript "m") (*6*) so that they can express their "foreignness" but cannot incorporate radioactive thymidine—the criterion of the response. Stimulation in the MLC test (division of responding cells)—the measure of foreignness—has occurred if the number of counts per minute of radioactive thymidine incorporated in an allogeneic mixture (such as, AB_m) is significantly increased as compared to that in an isogeneic control culture (such as, AA_m). Pairing of donor and recipient is accomplished by choosing the donor to whose cells there is the least response by cells of the recipient.

Studies in man suggested that the same genetic region controls the SD antigens and reactivity in MLC (*3*). In these initial studies of Amos and Bach, however, two siblings were studied who had inherited the same SD loci antigens [a finding that has since been confirmed with the much more sophisticated serology now available (*7*)], but whose cells did stimulate in MLC. One possible explanation given for this result (*8*) was that there was another locus closely linked to the HL-A SD loci, which could cause MLC activation but whose products were difficult to detect serologically—that is, an LD locus. The two siblings with identical SD loci would be different for the LD locus as a result of a recombinational event having occurred in one of the parents.

We now report evidence supporting the existence of LD differences in the MHC, which are not detectable serologically with the usual methods of immunization and testing but which can cause stimulation in MLC. Bailey *et al.* (*9*) have discovered a spontaneous mutation in the MHC of C57B1/6 mice. The mutant mice, H(z1), which are genetically identical (including serological identity at H-2K and H-2D) to C57B1/6 mice except for the mutation, reject skin from C57B1/6 mice and vice versa. It is not clear whether the mutation in the H(z1) mice is between H-2K and Ss-Slp, within the H-2K locus, or to the left of H-2K. Despite extensive immunization schedules, these workers have been unable to obtain agglutinating or cytotoxic antiserums against the "difference." There is stimulation in both directions in four separate MLC tests; one representative experiment, including cells of a third mouse strain differing from C57B1/6 and H(z1) at the MHC, is shown in Table 1, combination A. In each case

Table 1. Mixed lymphocyte culture results in various mouse strain combinations. Results are given as counts per minute ± the standard deviation. The *P* value was obtained by the *t*-test with the log of the transformed data, comparing each allogeneic mixture with the appropriate isogeneic control. Responding cells of one mouse strain (C57B1/6) were mixed with mitomycin C–treated stimulating cells of either the same strain (the isogeneic control cultures) or with cells of a different strain (for example, B10.D2, the allogeneic test strain). The cells were then incubated for several days, and radioactive thymidine was added to the cultures. If, in the mixture containing allogeneic stimulating cells, a significantly greater amount of radioactive thymidine was incorporated than in the mixture containing syngeneic stimulating cells, then stimulation had taken place.

Responding cells	Stimulating cells		
	C57B1/6	H(z1)	B10.D2
	Combination A		
C57B1/6	(5567 ± 516)	23756 ± 1191 $P < .001$	66305 ± 7512 $P < .001$
H(z1)	15545 ± 2496 $P < .001$	(5453 ± 494)	103279 ± 3567 $P < .001$
B10.D2	139685 ± 5501 $P < .001$	84931 ± 7211 $P < .001$	(3569 ± 650)
Responding cells	Stimulating cells		
	B10.A(2R)	B10.A(4R)	C57B1/10
	Combination B		
B10.A(2R)	(17338 ± 2060)	18585 ± 329 $0.2 < P < 0.4$	94587 ± 4007 $P < .001$
B10.A(4R)	47776 ± 224 $P < .001$	(10836 ± 888)	127642 ± 10608 $P < .001$
C57B1/10	103423 ± 18230 $P < .001$	90238 ± 7872 $P < .001$	(9861 ± 578)
Responding cells	Stimulating cells		
	AQR	B10.T(6R)	B10.A
	Combination C		
AQR	(3567 ± 1190)	32087 ± 1900 $P < .001$	7869 ± 439 $0.01 < P < 0.025$
B10.T(6R)	44181 ± 2071 $P < .001$	(3440 ± 1409)	52402 ± 4292 $P < .001$
B10.A	8425 ± 722 $0.1 < P < 0.2$	25823 ± 1583 $P < .001$	(6856 ± 1416)

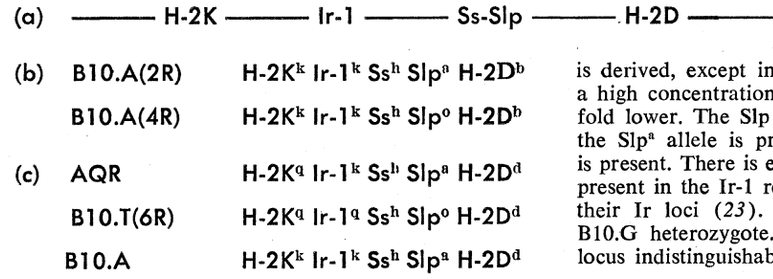

Fig. 1. Superscripts in this figure refer to the MHC chromosomes from which a given region is derived, except in the case of the Ss-Slp locus. The Ss^h allele specifies a high concentration of Ss protein in serum, the Ss^l allele an amount 20-fold lower. The Slp antigen is present only in males and then only when the Slp^a allele is present; the Slp antigen is absent when the Slp^o allele is present. There is evidence suggesting that more than one Ir locus may be present in the Ir-1 region. The B10.A(2R) and B10.A(4R) mice differ for their Ir loci (*23*). The B10.T(6R) strain was derived from a B10.A/B10.G heterozygote. AQR was derived from T-138(H-2^q), which is SD locus indistinguishable from B10.G.

a significantly higher number of counts per minute is incorporated into the allogeneic mixtures than into the control isogeneic ones. Thus, there is reciprocal MLC stimulation just as there is reciprocal skin graft rejection.

Direct evidence for the role of a locus (loci) between H-2K and H-2D in leading to MLC activation is gained from studies in two mouse strains, B10.A(2R) and B10.A(4R), which are genetically identical except for the MHC genotypes (*10*) (Fig. 1b). Cells of 4R mice respond to stimulating cells of 2R mice although there is no stimulation in the other direction, as shown in one of the five separate experiments giving these results (Table 1, combination B). Skin grafts between 2R and 4R mice are not rejected (*11*).

Additional evidence in this regard comes from studies in three other mouse strains, AQR (*12*), B10.T (6R) (*10*), and B10.A (Fig. 1c shows the MHC genotypes). AQR and B10.T(6R) mice differ for Ir-1 (*13*) and Slp (*14*) and are identical for H-2K and H-2D, but their cells stimulate in MLC (Table 1, combination C).

There are two possible interpretations for the reciprocal stimulation in the AQR-6R combination. (i) There is a gene or genes between H-2K and H-2D, and the product can lead to stimulation of the responding cells in MLC. (ii) Since AQR and 6R mice differ for minor histocompatibility loci as well as for the MHC, the minor loci differences cause the MLC stimulation. Arguments against this second possibility are the studies in which the response of the AQR cells to 6R stimulating cells and B10.A stimulating cells is compared. Cells of 6R and B10.A mice are genetically identical except for the MHC and thus differ from AQR by the same minor loci; yet there is repeatedly little or no MLC response of AQR lymphocytes to B10.A stimulating cells compared to the much stronger response of AQR cells to 6R stimulating cells. Whereas one might still argue that there is an interaction between the MHC and the minor loci in the AQR-6R mixtures, such a hypothesis requires further assumptions and thus seems less likely. Further, (AQR × C57BP/10)F_1 animals reject B10.T-(6R) skin (*14*), indicating that a difference in the MHC is responsible for skin graft rejection. The Slp antigen in the 2R-4R and the AQR-6R mice would seem an unlikely reason for the MLC stimulation since, at least in the AQR-6R combination, reciprocal one-way MLC tests are positive and the Slp antigen incompatibility exists in only one direction; further, male and female cells stimulate and respond in the 2R-4R combination. Extensive studies have tended to rule out the Slp antigen from a role in homograft reactions (*5*).

The suggestion by Amos and Bach (*8*) that there may be an LD locus linked to the SD loci in man has received support from the more informative family studies of Plate *et al.* (*15*), Yunis and Amos (*16*), and the extensive studies of Eijsvoogel, van Rood, and their colleagues (*17*). Nevertheless, these studies in man cannot be conclusive in establishing the existence of such loci in the MHC. Our studies in the mouse provide direct evidence for the existence of genetic differences in the MHC which lead to MLC activation and which cannot be defined serologically with the usual methods for immunization and testing. In addition, there are LD loci in the MHC, genetically separable from the SD loci, which can lead to MLC activation.

The results obtained in these studies raise several questions and demand a reevaluation of the MHC—a problem clearly and provocatively raised by the paper of Yunis and Amos (*16*). There are two questions to which we will address ourselves. First, what are the phenotypic products associated with LD differences, and second, what are the practical implications of these studies for the pairing of donor and recipient for transplantation?

In both the 2R-4R and the AQR-B10.T(6R) combinations described, the LD locus (loci) maps genetically with the Ir-1 locus. The Ir-1 gene product seems to be expressed in T lymphocytes, and it has been suggested that it controls a T cell receptor site (*4*). If the LD locus and Ir-1 locus are identical, then the receptors could function not only as recognition molecules on the responding cells but also as the foreign molecules on the stimulating cells. Any cell that has one or more Ir receptors which a second cell does not have might stimulate that second cell in MLC. In our study of a number of mouse strains in MLC the overall pattern of stimulation suggests that in several cases the greatest responses are associated with Ir region differences, whereas Ir region identity is at least sometimes associated with very weak stimulation (or none at all) in MLC. This will need confirmation by additional studies. It may be, in fact, that, although no cytotoxic or agglutinating antibody is produced against the receptors after immunization, a blocking antibody is synthesized (*18*).

Other explanations are possible. The LD locus product could be different from the Ir product. The LD product might be recognized by thymus-derived (T) lymphocytes, which are involved in cell-mediated immunity (graft rejection) and probably to a large extent in the MLC reaction, but which cannot stimulate those bone-marrow (B) lymphocytes that produce classical antibody. Alternatively, T lymphocytes may recognize differences in the spatial arrangement of the same set of antigens as defined serologically (*19*). The LD loci then could be considered "control" loci, which affect the surface patterns in which antigens are arranged. Last, there are loci in the MHC that affect susceptibility to viral infection (*20*); perhaps in a case such as the 2R-4R combination the 2R cells carry viral genes that lead to the difference recognized by the 4R cells.

Our findings provide possible practical implications for pairing of donor and recipient for transplantation. Genetic differences exist in the MHC, which are very difficult to detect serologically even though they can be the cause of rapid rejection of skin.

Although some studies have yielded evidence suggesting a correlation between pairing by typing for the HL-A antigens in man and graft survival (better survival given fewer antigenic differences), these studies have been somewhat disappointing (*21*). Similarly, the cells of a majority of unrelated individuals who appear to have identical SD antigens stimulate in MLC tests (*22*). Many possible reasons have been given for the lack of strong correlations; heterogeneity of the HL-A antigens, polyspecificity of the available serums, and our lack of knowledge concerning the strength of the different antigens have all been mentioned. Our findings suggest that one reason for the lack of a strong correlation may be the presence of LD loci—serologically undetected differences that can lead to MLC activation and skin graft rejection.

The H(z1) mutant suggests that in at least some cases the same mutation can affect both skin graft rejection and MLC activation; although one cannot be certain that only one gene is involved in this mutational event. While some LD differences may lead to skin graft rejection, others, such as the 4R-2R, do not. In the 4R-2R combination there is stimulation in only one direction which is a very unusual finding using normal allogeneic cells; further experiments will be needed to study the relation of graft rejection to LD differences and MLC activation. At the present time studies with MLC tests, serological detection of antigens, and skin graft studies (where possible) are needed to gain a more complete understanding of the MHC, the factors important for MLC activation and those important for graft survival.

FRITZ H. BACH
MICHAEL B. WIDMER, MIRIAM SEGALL
Departments of Medical Genetics and Medicine, University of Wisconsin, Madison 53706

MARILYN L. BACH
Departments of Pediatrics and Pharmacology, University of Wisconsin, Madison

JAN KLEIN
Departments of Oral Biology and Human Genetics, University of Michigan, Ann Arbor

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Specific Inhibition of Plaque Formation to Phosphorylcholine by Antibody against Antibody

Abstract. *Spleen cells from BALB/c mice immunized with heat-killed rough pneumococci (strain R36A) or spleen cells from normal mice immunized in vitro with the same antigen produce direct hemolytic plaques against sheep erythrocytes coated with pneumococcal C polysaccharide or conjugated with phosphorylcholine. Formation of plaques is specifically inhibited by phosphorylcholine or by antiserum to mouse immunoglobulin A myeloma protein which binds phosphorylcholine. Thus, the myeloma proteins and normal BALB/c antibodies share similar idiotypic determinants. This experimental system is suitable for probing the role of the antigen receptor in the immune response.*

Myeloma proteins obtained from mice carrying plasma cell tumors have detectable antibody activity against chemically defined haptens such as phosphorylcholine (*1*). Potter and Lieberman have reported the preparation of antiserums to idiotypic determinants in BALB/c myeloma proteins which bind phosphorylcholine (*2*). For convenience, the myeloma-specific determinants have been referred to as "idiotypes" and the antiserums as "anti-idiotypic serums" (*3*). To test the biological activity of the anti-idiotypic serums, we devised the following methods: (i) an antibody response specific for phosphorylcholine was regularly induced by immunizing BALB/c mice or cultures of spleen cells with heat-killed pneumococci; and (ii) the plaque-forming cell (PFC) technique was modified to demonstrate single cells synthesizing antibody to phosphorylcholine. We find that the anti-idiotypic serums inhibit formation of plaques; apparently the idiotypic determinants in myeloma proteins which bind phosphorylcholine and those in antibodies produced in a primary response to pneumococci are identical or very similar.

Purified monomeric IgA myeloma proteins obtained from BALB/c mice carrying the TEPC-15 or the HOPC-8 tumors were donated by Dr. M. Potter. Antiserums against these myeloma proteins which bind phosphorylcholine were prepared in A/He mice (*2*). The antiserums were absorbed with normal BALB/c serum; they reacted only with those myelomas which bind phosphorylcholine and share specific antigenic determinants (*2*).