short, microvilli appear in regions of red cell contact, and with increasing time (2 to 3 hours) the microvilli become numerous, long (up to 6 μm), undulating, and branched (Fig. 2). Indeed, at this stage, rosetted Molt-4 cells exhibit a surface topology barely distinguishable from that of "B-type" lymphocytes (*1–3*). However, the regions with abundant long microvilli occur only near sites of contact with SRBC; other regions of the lymphoid cell periphery may continue to be quite bald. Moreover, we do not observe the drastic distortion of erythrocyte surfaces that characterizes complement-dependent B-cell rosettes (*1*). In contrast, unrosetted Molt-4 cells, as well as cells maintained for up to 3 hours under rosetting conditions but without SRBC, exhibit no change in surface topology.

In more than 90 percent of more than 500 rosetted Molt-4 cells counted in four series, the lymphocytes' surface topologies were clearly distinguishable from the resting state. Furthermore, the contacts between Molt-4 cells and SRBC are mediated by microvilli, as reported before for conventional rosetting systems (*1*). In general the microvilli interact mostly with the edges of the rosetted erythrocytes, but very complex contacts can be observed. Quite frequently, for example, the lymphocyte microvilli seem to grasp the erythrocytes in tentacle fashion.

Contact between SRBC and Molt-4 cells induces a dramatic increase in the number, lengths, and complexity of the lymphocytes' surface microvilli. It seems reasonable to assume that contact with the erythrocytes produces an initially localized, but vigorous increase in surface rearrangement. The resulting extensive microvilli produce a lymphocyte morphology very similar to that seen in B-rosettes (*1*), and we therefore suggest caution in attempts to classify T- and B-cells on the basis of morphologic criteria alone.

If Molt-4 cells constitute an adequate T-cell model, our findings can explain the curious variability of lymphocyte surface topology reported for T-cell rosettes (*1*). The number and lengths of microvilli increase (within limits) with the duration and extent of red cell contact; neither of these environmental variables is usually well defined.

However, we suspect more fundamental implications and propose that the bald and hairy surface topologies of lymphocytes represent separate stages of differentiation. Accordingly, the bland surface features of native thymocytes and freshly cultured Molt-4 cells would reflect a quiescent state. Contact with appropriate surfaces, such as with erythrocytes on exit from the thymus, then induces a surface activation manifested through the development of elaborate microvilli. The biochemical and immunological correlates of the surface activation phenomenon must be explored in order to establish its biological significance.

PECK-SUN LIN
DONALD F. HOELZL WALLACH
Department of Therapeutic Radiology, Division of Radiobiology, Tufts–New England Medical Center, Boston, Massachusetts 02111

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Genetic Mapping of Ir Locus in Man: Linkage to Second Locus of HL-A

Abstract. *Fifty-seven members of a family that spanned three generations were studied for antigen E and ragweed skin sensitivity and HL-A antigens. There was significant association between the haplotype HL-A 2-12 and antigen E skin hypersensitivity ($F = .22$ to $.26$) in this family. The map order is first locus of HL-A, second locus of HL-A, and IrE. These determinants are considered to be part of the linkage group HL-1.*

The histocompatibility complex of man, HL-1, includes genes determining certain leukocyte and tissue (HL-A) antigens and the ability to stimulate in mixed lymphocyte culture (MLR-S) (*1*). It is here proposed that HL-1 includes a determinant for hypersensitivity to ragweed antigen E (IrE) and the map order is the first locus of HL-A, second locus of HL-A, and IrE.

This report is based on studies of 57 subjects of a large Minnesota family. Thirteen members of the B. family had histories of respiratory symptoms of asthma or rhinitis (or both) during the ragweed season in Minnesota (August through Sepember). Intradermal tests were performed with 1 : 500 dilutions of ragweed (Hollister-Stier) (*2*). Antigen E, a purified protein derived from ragweed pollen extract, was used for skin testing at concentrations from 1×10^{-12} to 1×10^{-1} mg/ml, and the end point dilution with a positive reaction was scored. The skin tests were read for immediate wheal and flare reactions at 20 minutes. They were graded on a 0 to 4+ scale (*3*). A significant positive reaction was considered to be 2+ or greater. The antigen E preparation (*4*) gave negative reactions in 14 normal, nonatopic individuals ranging in age from 14 to 40 years with dilutions of 10^{-12} to 10^{-1} mg/ml (for all tests 0.02 ml of each dilution was used). Forty-eight ragweed-sensitive individuals ranging from 18 to 48 years of age gave positive reactions to the antigen E preparations, with end point dilu-

Table 1. Estimated frequency of recombination between loci for ragweed sensitivity and HL-A.

Parent phenotype	F*	S.E.	P†	Sample size
HL-A 2 + IrE (a)	.26	.08	$<.05$	27
HL-A 12 + IrE (b)	.22	.08	$<.01$	27
HL-A 3-W18 + IrE (c)	.89	.10	$<.05$	9
HL-A 9 + IrE (d)	.71	.12	$>.10$	14
W15 + IrE (e)	.79	.11	$<.10$	14
HL-A 9-W18 + IrE (f)	.60	.09	$>.10$	5

* Observed frequency of recombinants in offspring. † Probability under the null hypothesis of no linkage between HL-A and IrE. $P < .05$ and $F < .5$ indicate (lines a and b) that a particular HL-A haplotype is linked to the allele for ragweed sensitivity (IrE) (coupling); $P < .05$ and $F > .5$ (line c) indicate that HL-A is linked to a different allele at the locus for IrE sensitivity (repulsion); and $P > .05$ indicates independent segregation (lines d, e, and f).

tions from 10^{-2} to 10^{-10} (4 individuals with end point reactions at 10^{-2} to 10^{-4} mg/ml, 19 with end point reactions at 10^{-5} to 10^{-7} mg/ml, and 25 with end point reactions at 10^{-8} to 10^{-10} mg/ml).

Determinations of the HL-A antigens were performed on lymphocytes obtained from the peripheral blood by a two-stage lymphocytotoxicity method (*5*). Assignment of haplotypes was based on inheritance patterns within the family (*6*). Red cell antigens were studied to exclude mixed paternity. Half siblings III-1 and III-2 (Fig. 1) were known to have different fathers. The fathers of III-1 and III-33 were not available for study. Data on the B. family presented in Fig. 1 include clinical history of ragweed asthma or hay fever (or both), age, HL-A haplotypes, and skin reaction to ragweed and antigen E. Antigen E end point dilutions producing a positive skin test ranged from 10^{-3} to 10^{-9} mg/ml (Fig. 1).

Apparent recombination frequency between HL-A and antigen sensitivity is presented in Table 1. Since HL-A is usually inherited as a single genetic unit, it is usual to designate HL-A in terms of haplotypes rather than as antigenic specificities. However, the family includes an informative recombinant within the HL-A region. This individual, subject III-15, inherited specificity HL-A 9 from the maternal HL-A 9-W15 haplotype and HL-A 12 from the paternal haplotype HL-A 2-12. The association of HL-A to antigen E is therefore recorded in relation to the determinants of each of the four HL-A specificities involved in the recombination: HL-A 2, 9, and 12 and W15. Since the HL-A haplotypes HL-A 3-W18 and HL-A 9-W18 are not involved in recombination, they are treated as a unit. The only significant linkage between ragweed hypersensitivity and HL-A is with haplotype HL-A 2-12. Subjects I-1 (age 73), II-7 (age 46), II-11 (age 42), III-17 (age 14), III-21 (age 20), and III-24 (age 9) gave negative skin hypersensitivity reactions to ragweed or antigen E (or both) and have the haplotype HL-A 2-12. Thus there is full penetrance of IrE in 11 of 17 individuals (65 percent) carrying the HL-A antigen 12. Subjects II-1, II-3, II-5, and II-11 are not included in the linkage analysis because the children of a negative parent are considered not to have inherited the determinant for hypersensitivity.

There is a maximum of seven possible HL-A IrE recombinants in this family. One of these, III-15, inherited an HL-A recombinant resulting from crossover between the HL-A 9 determinant from the paternal 9-W15 and the HL-A 12 determinant from the IrE linked HL-A 2-12 haplotype. The other six are HL-A IrE recombinants: subject II-11 inherited the 2-12 haplotype but is negative for antigen E or ragweed skin hypersensitivity, and all her eight children including five with haplotype 2-12 are normal; III-36 received the IrE locus by crossover from the paternal HL-A 2-12 to the paternal HL-A 9-W15 haplotype. The remaining four are only possible recombinants: III-17, III-21, and III-24 are 9, 14, and 20 years of age, respectively,

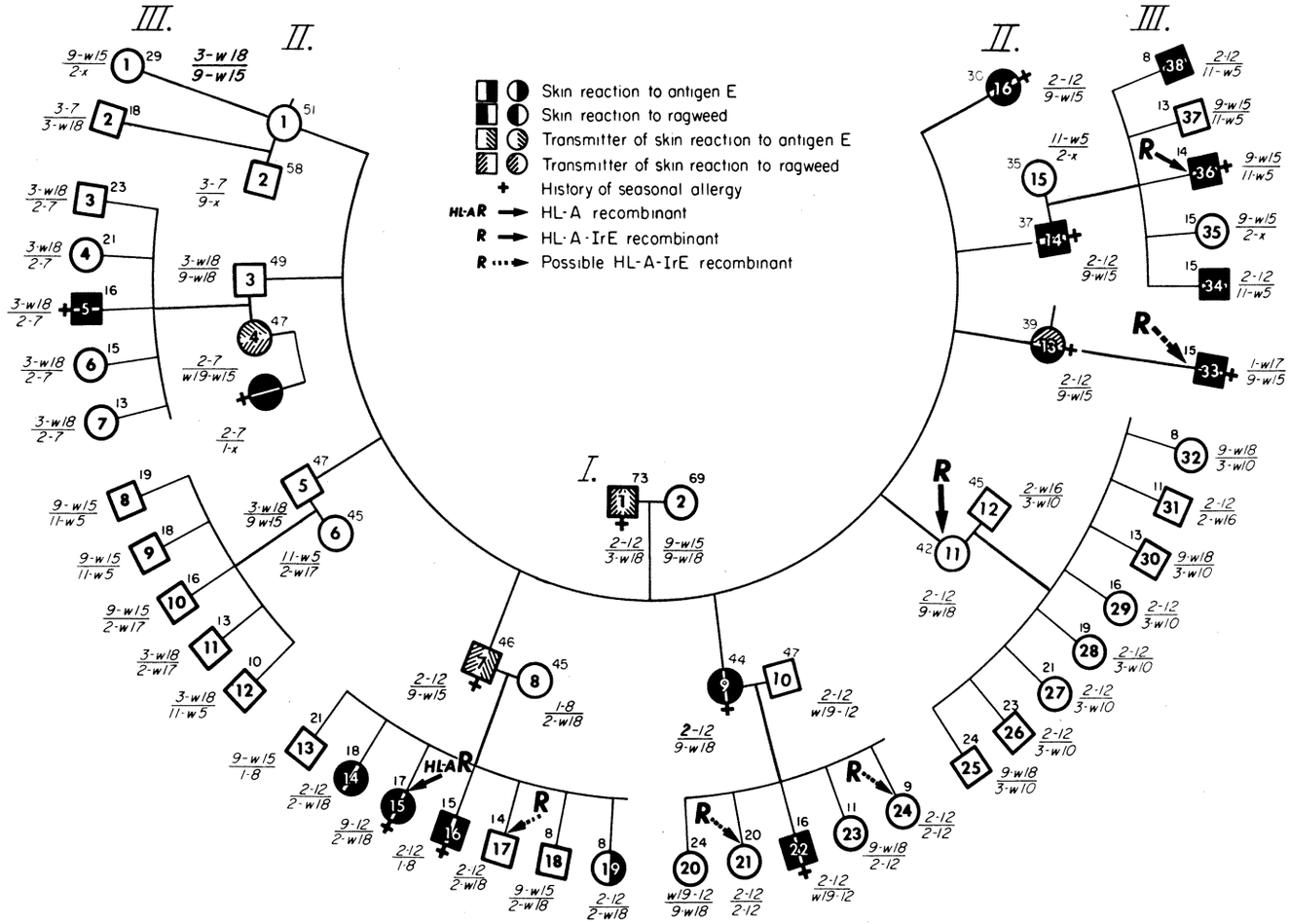

Fig. 1. Linkage of HL-A haplotype and IrE in the B. family. The numbers inside the squares and circles identify the subjects. The numbers above the squares and circles are the ages of the subjects.

and have no Ir locus demonstrable by genetic transmission or skin testing. The fourth, III-33, does not possess haplotype 2-12 but his 9-W15 haplotype might have received the IrE from the maternal HL-A 2-12 haplotype. However, the lack of data on his father leaves the possibility of inheritance of IrE from the paternal haplotype.

A second example of hypersensitivity to ragweed and antigen E was introduced by II-4 and presents an apparent low penetrance form of hypersensitivity. Subject II-4 had no evidence of hypersensitivity although her sister did. Of the five HL-A identical children of II-3 and II-4, only one had clinical and cutaneous hypersensitivity. There is no suggestion of linkage to HL-A in this segment of the family.

Immediate reaginic immune response to ragweed pollen has a high familial incidence (*7*). Levine *et al.* have established a relation between hypersensitivity to antigen E or ragweed and the distribution of HL-A histocompatibility antigens within a family (*8*). This suggested a genetic basis for the hypersensitivity and linkage between the genes for hypersensitivity and for HL-A. Buckley *et al.* have shown that the capacity to respond to a variety of biological products is also linked to the HL-A system (*9*).

Our data are consistent with linkage between the locus for HL-A 12 and the IrE locus or loci controlling hypersensitivity to ragweed antigen E with a map distance of 7 to 22 crossover units, the best fit being realized when IrE is placed adjacent to the determinant for the second locus specificity HL-A 12. This assumption is based on the reaction of III-15 who has clinical allergy and skin hypersensitivity to antigen E and ragweed. Since she inherited a recombinant HL-A haplotype which includes HL-A 12, it appears that the IrE gene was inherited with the second locus of HL-A. In addition, it is noteworthy that the MLR-S gene of III-15 is inherited with the HL-A antigen 12 (*10*).

To establish whether or not the loci determining HL-A and ragweed sensitivity are in fact linked, we determined the maximum frequency of individuals who could be considered to be recombinants. This frequency is 6/27 or .222 [standard error (S.E.) = .08] with HL-A 12 and 7/27 or .259 (S.E. = .08) with HL-A 2. Therefore we can reject the hypothesis of independent segregation ($P < .05$) and consider these traits linked (Table 1). To establish whether or not the locus for ragweed sensitivity is inside the locus for HL-A, we estimated the minimum frequency of recombinants observed. Therefore, we eliminated III-17, III-21, and III-24 because they are 14, 20, and 9 years of age and have no Ir locus that is demonstrable by genetic transmission. We might also eliminate III-33 because no testing was done on his father or on his family. Considering these individuals as nonrecombinants, we obtain a minimal estimate of linkage of 2/27 or .07 (S.E. = .05) for HL-A 12 and 3/27 = .11 (S.E. = .06) for HL-A 2. Since the recombination frequency within the HL-A locus is estimated to be at most .01 (*11*) (S.E. in a sample of 27 = .02), one can reject the hypothesis that the locus determining ragweed hypersensitivity is between the two loci determining HL-A ($P < .05$).

It is known from previous studies that the MLR-S gene also maps outside HL-A in association with the second locus determinant (*1*). Therefore the probable map order is either HL-A first locus, HL-A second locus, MLR-S and IrE or HL-A first locus, HL-A second locus, IrE, and MLR-S. Since the map distance between the first and second locus determinants for HL-A is somewhat less than 1 crossover unit, IrE, the locus for production of a reaginic antibody, must lie outside the HL-A complex proper.

M. N. BLUMENTHAL
Department of Medicine, Box 194 Mayo, University of Minnesota Hospitals, Minneapolis 55455

D. B. AMOS
Division of Immunology, Box 3010, Duke University Medical Center, Durham, North Carolina 27710

H. NOREEN
Department of Laboratory Medicine and Pathology, Box 198 Mayo, University of Minnesota Hospitals

N. R. MENDELL
Division of Immunology, Box 3010, Duke University Medical Center

E. J. YUNIS*
Department of Laboratory Medicine and Pathology, Box 198 Mayo, University of Minnesota Hospitals

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