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4. Phased correlation as described here requires, in addition to the computer inputs illustrated in Fig. 1, an input which marks the beginning of each stimulus cycle. Before the data are processed the period of the stimulus cycle is measured so that it can be divided into phase bins, just as for conventional single unit cycle histograms.
5. By focusing on the response at a particular conduction velocity under a number of stimulus conditions (such as static bias angle, frequency, or rate of rotation), one may deduce the repertoire of responses of a particular unit. We assume that the response of a given unit is principally within two or three bins, depending on signal to noise ratio. Since each bin is 25 μsec in width, the nominal resolution is 75 μsec. Initially we were surprised that such a small number of units were active under a given set of conditions. The viability of the MAN was tested by observing that units present at one electrode were also present at the other and by observing the size of a compound action potential generated at one site and recorded at the other. We conclude that only a small fraction of the units (perhaps 10 of 75) respond under a particular set of stimulus conditions.
6. Cycle histograms or poststimulus histograms may be obtained by collecting the contributions at a particular conduction velocity (two or three adjacent bins) and plotting the contribution against the time of occurrence within the stimulus period.
7. The velocity resolution obtainable is directly related to the signal to noise ratio and the electrode separation. Improved resolution could be obtained by increasing either of these factors. In the application described here it is usually possible to restrict the number of active units to a manageable size by controlling the stimulus conditions. Phasic units can be eliminated by using low-frequency stimuli, for example. Different groups of units are recruited at different static bias angle combinations. The total spatial-temporal response of the MAN can be deduced by observation and collation of the restricted data sets.
8. The results shown in the inset in Fig. 2B indicate that these are probably four distinct units. This is most clear in the case of *a* and *c*, where all traces seem to superimpose with one amplitude value. Responses *b* and *d* are less clear, but are thought to result from single units. Although these results are probably no more equivocal than most results obtained in single unit studies, we think this problem needs more work. On occasion we have observed pairs of tonic units that had similar conduction velocities and similar response patterns. The existence of two units with nearly identical conduction velocities and action potential shapes is revealed by careful examination of their responses under varying stimulus conditions. It is usually possible to selectively recruit only one unit of the pair by manipulation of the stimulus conditions. Under most conditions they seem to operate as a "functional unit." Improvement of the resolution of our technique may allow separation of such responses. A separate, but related, problem involves two or more action potentials triggering one event. When nearly simultaneous occurrence of action potentials at one of the electrodes takes place, information is lost. We have found this to be rare (1 in 10,000 for the data shown), and the effect is distributed uniformly over the units under study.

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HL-A Linked Antigen E Immune Response Genes: An Unproved Hypothesis

Levine *et al.* (*1*, *2*,) and Blumenthal *et al.* (*3*) have presented data from which they suggest that there is genetic linkage between HL-A and responsiveness to the ragweed pollen allergen, antigen E. We do not question the data of these authors but seriously question the interpretations. There are standard methods for linkage analysis in families (*4*, *5*) which were not used by either Levine *et al.* (*1*, *2*) or Blumenthal *et al.* (*3*). We wish to raise the following points.

Concerning the report of Levine *et al.* only one out of a total of 14 allergic families interviewed was reported in detail. Seven of the interviewed families were not studied because hay fever was manifested only in the propositus. A more detailed analysis of all families would have enabled the reader to evaluate all the evidence. Levine *et al.* believe that they have demonstrated linkage between IgE (immunoglobulin E) mediated skin sensitivity to antigen E and a particular familial HL-A haplotype in the seven families specially selected for "expressivity" of ragweed hay fever. Seven families, of a size sufficient for these authors to make a positive statement for linkage, should be amenable to conventional linkage analysis by a method such as that of Morton (*4*); this was not attempted.

Levine *et al.* discussed multifactorial inheritance of IgE mediated skin sensitivity to antigen E, but they did not fully consider how the presence of "unexpressed" Ir–antigen E (Ir-AgE) genes might affect linkage analysis in the seven families actually selected for study.

The statistical methods used to analyze for "linkage" (*1*, table 2) and to determine the prevalence of Ir-AgE genes (*1*, reference 13) are not stated clearly. It is not obvious how selected subjects (entering an allergy clinic) can be used to ascertain the population frequencies of Ir-AgE genes. Nevertheless, assuming that their estimate of 0.4 for the combined frequency of all such genes is approximately correct, that estimate must be used in the linkage analysis to calculate the probability that unaffected people possess an Ir-AgE gene (in the homozygous or heterozygous state) or that affected individuals are homozygous for the Ir-AgE gene.

In the family reported in detail by Levine *et al.*, the spouses of subjects 1, 3, 4, and 15 apparently were not studied. The data were not reported in table 1 (*1*) and were not further clarified in a subsequent publication (*2*). One cannot assume that a common trait such as possession of an Ir gene for antigen E might not be present in these people (see above). In this case, sensitivity to antigen E in their offspring, Nos. 5, 6, 7, and 16, might easily have been inherited from the unstudied parent whether or not he or she "expresses" specific allergy. On the one hand, there is the problem of the probable high frequency of Ir-AgE genes and, on the other, the differential expressivity in IgE mediated skin responsiveness in different family members. This problem is difficult to analyze if the mating types cannot be unequivocally identified, and analysis is impossible when only one parent is studied. For example, if subject 3 and her spouse both carry a single Ir gene, the mating is an intercross; only homozygous offspring from any intercrosses are informative for linkage. In this case, homozygous Ir-AgE positive individuals cannot be distinguished from heterozygotes; and, further, the homozygous negatives cannot be distinguished from unexpressed positive individuals. Consequently, this pedigree cannot be analyzed for linkage.

Subjects 3 and 8 (mother and daughter), who have the HL-A 1,8 haplotype

but showed a negative skin test to antigen E, were said to have "trace amounts" of IgG (immunoglobulin G) antibody to antigen E in their serums and thus have an Ir-AgE gene. The radioimmunoassays for IgG antibody were performed with ragweed fraction D which contains only about 19 percent antigen E (*6*). It cannot be ruled out that the IgG antibody was against some other component of fraction D, especially since antigen E is quite difficult to label by the standard chloramine-T procedure. It should also be pointed out that IgG antibody was studied only in six carefully selected members of the one family reported in detail.

On the basis of their data, we do not believe that Levine *et al.* have actually demonstrated linkage between HL-A and an Ir gene controlling IgE antibody response to antigen E, nor that IgE and IgG responsiveness to antigen E may be under single gene control.

The report of Blumenthal *et al.* attempted to demonstrate genetic linkage between an HL-A 2,12 haplotype and an Ir–antigen E gene in a kindred of 57 people. Of 21 people having the putative Ir–antigen E (abbreviated IrE in their report) linked HL-A haplotype, 9 (43 percent) are reported to have a positive skin test to antigen E.

Blumenthal *et al.* did not clearly indicate either the source and purity of the antigen E preparation or the concentration of ragweed pollen extract used for skin testing (*7*). They classified as positive those subjects who required as much as 1 μg of antigen E per milliliter to produce a significantly positive (2+) intradermal skin test, without giving further quantitative data on family members. If a more dilute endpoint for 2+ reactivity had been used, one wonders how many subjects with the HL-A 2,12 haplotype would fall into the insensitive category (*8*). It is important to note that, because of the exquisite sensitivity of the skin test assay, reactions to as much as 1 μg of antigen E per milliliter are questionable even when highly purified antigen E is used, because such responses may well be due to impurities. This is especially likely if the authors used a commercial preparation of antigen E, inasmuch as these are sometimes less than 90 percent pure. Furthermore, subject III-19 is recorded as showing a positive skin test to antigen E but a negative test to crude ragweed extract. This is an anomalous finding, unless the extract is denatured or too dilute, since antigen E is a major component in crude ragweed (*6*).

The unproved assumption that HL-A is closely linked to an IrE gene is taken by Blumenthal *et al.* as established fact. People not fitting into this hypothesis are classified as "HL-A–IrE recombinants" or "transmitters of skin sensitivity to antigen E." For example, II-11 is designated as a recombinant because, even though she possesses the HL-A 2,12 haplotype, she is antigen E negative and her five children who inherit that haplotype are also negative. In human genetics, as in the genetics of lower organisms, the detection of a recombination requires the presence of identifiable markers on either side of the crossover point. For the IrE gene itself to be a suitable marker, a means for unambiguous identification is necessary.

The hypothesis of Blumenthal *et al.* of linkage between IrE and HL-A 2,12 in this family is completely dependent on the grandfather, I-1, and his son, II-7, being "transmitters." Both of these subjects are reported to have seasonal allergy and, thus, have genes required for the general expression of allergy. They possess the putative IrE linked haplotype yet have negative skin tests to antigen E and to crude ragweed. This circumstance seems unlikely if Blumenthal *et al.* wish to hypothesize a single Ir gene locus.

Blumenthal *et al.* suggest a recombination frequency of more than 20 percent between HL-A 12 and IrE. This conclusion was reached after the large family of II-11 and her offspring were eliminated from the analysis. Furthermore, the analysis was for association not linkage. If a recombination fraction as large as 20 percent actually exists between these two loci, it would be difficult to consider placing IrE within the major histocompatibility complex.

We conclude that the Blumenthal *et al.* need to provide definitive experimental evidence for their hypothesized "transmitters" and "recombinants" before further evaluation of genetic linkage between IrE and HL-A is possible.

The apparent homology of HL-A and the major histocompatibility complexes (MHC) of experimental animals and the evidence for Ir genes within the MHC of these animals suggest their existence in a syntenic relationship with HL-A in man. The syntenic relationship is obvious in mice primarily because of the availability of inbred and congenic strains, but would be expected to be difficult to establish in polymorphic outbred species such as man, since immune response is a multifactorial trait (*9, 10*). It is understandable that both Levine and Blumenthal and their collaborators would wish to establish HL-A linked Ir genes in man as we, ourselves, have attempted to do.

In studies of large populations of highly allergic people, we have found a highly significant association between possession of the HL-A 7 or an antigen of the HL-A 7 cross-reacting group and IgE and IgG antibody responses to ragweed pollen allergen Ra5 (*11*). This allergen has a known primary structure consisting of 45 amino acids and a molecular weight of 5,000 (*11, 12*). We also found a significant association between IgE mediated sensitivity to the more complex allergen, rye group I (molecular weight, 27,000), and antigens of the HL-A 8 cross-reacting group (*10, 13*), which is especially striking in allergic subjects having low levels of total IgE in their serums. We could find no association between sensitivity to antigen E (molecular weight, 38,000) and a specific HL-A type even in people with low levels of total IgE. This suggests that there may well be many different Ir genes for antigen E, as well as for components AgK and BPA-R known to be related immunologically to antigen E (*14*), which makes antigen E a rather poor choice for genetic studies (*10*).

In our studies of specific IgE mediated skin sensitivity and serum IgG antibodies in 31 local allergic and 23 large Pennsylvania Amish families, we did not observe concordant transmission of a particular familial HL-A haplotype and specific response to any one of several highly purified pollen allergens, including antigen E (*15*). Ten of these families were also studied for lymphocyte responsiveness to allergen in vitro to try to obtain a more direct assay of T-cell response (*16*). Again, we did not observe linkage with HL-A, suggesting that control of such responsiveness may itself be genetically complex.

As in the studies of Levine *et al.* and Blumenthal *et al.*, we find many failures of transmission from parent to child of specific responsiveness (measured by any one or a combination of our assays), as well as discordance between sibs for specific response

and HL-A haplotype. In allergic families, we more commonly see a high degree of concordance between specific IgE mediated skin sensitivities in allergic siblings who have high levels of total IgE in their serums (*17*). In such family members, and in unrelated populations, genetic regulation of the IgE level is observed as obscuring the effect of specific Ir genes. The IgE regulating gene appears to be just one of the multiple genetic factors affecting expression of the postulated HL-A linked Ir genes, and the problem is further compounded by differences in environmental exposure to allergens (*9, 10*).

At the present time, we believe that neither our extensive family data nor the data of Levine *et al.* and Blumenthal *et al.* are sufficiently clear indications of linkage between Ir genes and HL-A.

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Bias and Marsh state that they would have liked to see the detailed data on all 14 families that entered our study (*1*). As we reported, only the seven families (out of 14) that had ragweed hay fever in successive generations were studied. In the other seven families, only one person had the disease. These families were not studied, as we were interested in the inheritance of the disease as well as of certain immunological parameters—that only about half of our patients with hay fever have family members with the disease is a common finding in our experience. Our original manuscript contained detailed information on three of the seven families studied. The editors asked that the detailed data of the two smaller families be removed in order to save page space. However, the pertinent data for seven families were placed into our table 2 (*1*).

Our conclusion of linkage is based on the data in table 2. Our method was simply comparing the two haplotypes of the propositus in blood relatives of the propositus with regard to occurrence of high avidity IgE antibody to antigen E and clinical ragweed hay fever. Eliminating the propositus from the calculations, and then testing the hypothesis that one haplotype was associated with ragweed pollinosis and the other was not, we found that 13 of 19 blood relatives of the propositus' blood carrying the ragweed-associated haplotype had ragweed hay fever (and strong wheal-and-flare skin reactivity to dilute soutions of antigen E) compared to none of 11 blood relatives of the propositus who carried the other haplotype of the propositus. This difference is statistically significant. These association data indicate genetic linkage. The advantage of comparing the two haplotypes of the propositus in this way is to randomize genetic information contained in other chromosome pairs. Other data in our report concerned immunological specificity of the indicated Ir–antigen E genes, and expression of these genes to include IgG antibody formation, properties that permit comparison to Ir genes in inbred mice.

We suggested that other genetic factors may operate to permit expression of the Ir–antigen E genes, in order to explain why hay fever is common in successive generations of some families, while only occurring sporadically in other families. We suspect that these other hypothetical genetic factors may operate (either directly or indirectly) to control the permeability of allergens (of certain physical sizes and properties) through respiratory mucous membranes. However, no direct studies on these factors are available as yet.

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Locus Coeruleus Lesions and Learning

Crow (*1*) has proposed that the ascending noradrenergic system originating in the locus coeruleus mediates an essential reinforcement component of learning. Anlezark *et al.* (*2*) reported that bilateral lesions of the locus coeruleus in rats deplete cortical noradrenaline and "markedly impair or even abolish" increases in running speed for food reward in an L-maze. If locus coeruleus lesions disrupt an animal's learning capacity as the authors concluded, this deficit should appear in tasks with different performance criteria, for example, an increase in correctly discriminated responses. We investigated the effect of locus coeruleus lesions on the performance of animals in a T-maze discrimination.

Male Holtzman rats with bilateral one-stage or bilateral two-stage lesions of the locus coeruleus were reduced to 85 percent of peak body weight by food deprivation at least 3 weeks after the last operation (*3*). The animals were introduced to a straight runway