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7 April 1969

Human Leukocyte Antigenic Specificity HL-A3: Frequency of Occurrence

Abstract. *Reactivity of antiserum against HL-A3, a human leukocyte and tissue antigenic specificity, depends upon a property of the lymphocyte as well as on the potency of the serum. Many reactions of HL-A3 antiserums can only be recognized through absorption or by a two-stage test not in general use. Interpretations of donor-recipient compatibility and of the constitution of HL-A alleles are affected by these findings.*

Formal designations have been given to six specificities of the HL-A system of human leukocyte and tissue antigens (*1*). These factors are recognized by serums which have been shown, through absorption, to be oligo- or monospecific (*2*). Other serums which give comparable but not identical reactions to these "type specific" serums are quite commonly found. Additional reactions are frequently due to the presence of a second antibody. Where two antibodies are present in a serum, either component can be removed, leaving the other intact. With certain serums, selective absorption has not been possible, and cross-reactivity between antigens has been invoked as an explanation (*3*). We have had occasion to study a subject, BC, whose serum reacts with cells that carry HL-A3 specificity (positive) and also with cells generally considered to be HL-A3–negative. The reaction with HL-A3–negative cells does not appear to be a simple cross-reaction, and the various samples of BC serum subjected to absorption appear to be monospecific. This suggests that the HL-A3 specificity is more widely distributed than supposed. We now suggest that there are wide variations, genetically determined, in the amount of HL-A3 antigen available on the lymphocyte surface.

Subject BC was immunized against lymphocyte donor MF on four separate occasions over an 18-month period by intradermal injection of 5×10^6 lymphocytes at each of ten sites. Two series of injections spaced 7 weeks apart were needed to initiate the production of cytotoxic antibody; thereafter one injection induced a rapid rise in antibody titer (Fig. 1). Samples obtained at intervals were titrated against lymphocytes from a panel of unrelated donors in a simple cytotoxity test (*4*).

Cells from seven subjects were distinguished by their greater reactivity with all serum samples. These were grouped as class 1. Cells from a second group (class 2) of subjects consistently reacted at a lower titer and failed completely to react with several of the serum samples. Cells from a third donor class (class 3) reacted only with an undiluted serum. Most members of this class failed to exhibit any reaction with weaker serums. A few other reactions appeared among class 3 cells in a more sensitive assay system in which the cells were washed after incubation with antibody and before exposure to complement (*4*). A fourth class of cell could be distinguished in that they reacted only in this two-stage test. The four major categories were clearly distinguishable from each other, but within each category there was a certain amount of variability. This is indicated by the ranges of reactivity represented in Fig. 1, and by the frequency of reaction of the most sensitive cells within a class with certain serums (Table 1). The same reaction groups were found when cells from the panel were tested with antiserums to HL-A3 (*5*). Various samples of these serums covered a range of reactivity comparable to that obtained with different samples of BC serum (Table 1). The frequency of reactivity

Table 1. Cytotoxicity reactions of antiserums to HL-A3 including 12 bleedings of a single subject (BC). Samples from BC were obtained during four immunizations with the lymphocytes of another human subject (MF).

Serum identification*	Class 1†	Class 2	Class 3		Class 4		Frequency‡ (%)
			One-stage	Two-stage	One-stage	Two-stage	
Antiserums to HL-A3 from serum bank							
2506-03-01-03	7/7	5/5	6/10	6/10	0/4	2/4	58
2506-02-22-01	7/7	1/5	0/10	1/10	0/4	0/4	22
2506-02-17-01	6/7	0/5	0/10	0/10	0/4	0/4	17
2506-03-09-02	7/7	5/5	1/10	1/10	0/4	0/4	36
2506-03-22-02	7/7	5/5	0/10	4/10	0/4	2/4	50
1017-02-07-03	7/7	5/5	2/10	6/10	0/4	3/4	58
2506-06-22-01	7/7	5/5	0/10	0/10	0/4	0/4	33
BC serum							
6-16-65	7/7	0/5	0/10	1/10	0/4	0/4	22
7-1-65	7/7	1/5	0/10	1/10	0/4	0/4	25
10-15-65	7/7	5/5	0/10	3/10	0/4	1/4	44
11-11-65	7/7	3/5	0/10	0/10	0/4	0/4	28
3-9-66	7/7	5/5	9/10	10/10	0/4	4/4	72
3-15-66	7/7	5/5	5/10	8/10	0/4	0/4	58
3-24-66	7/7	5/5	1/10	1/10	0/4	0/4	36
6-14-66	7/7	0/5	0/10	0/10	0/4	0/4	19
10-5-66	7/7	0/5	0/10	0/10	0/4	1/4	22
11-22-66	7/7	5/5	10/10	10/10	0/4	4/4	72
11-27-66	7/7	5/5	10/10	10/10	0/4	0/4	61
2-2-67	7/7	5/5	0/10	0/10	0/4	0/4	33

* NIH catalog number or bleeding date. † Proportion of positively reacting samples that were obtained from donors from classes 1 to 4 (see text). ‡ Including positive reactions in the two-stage test.

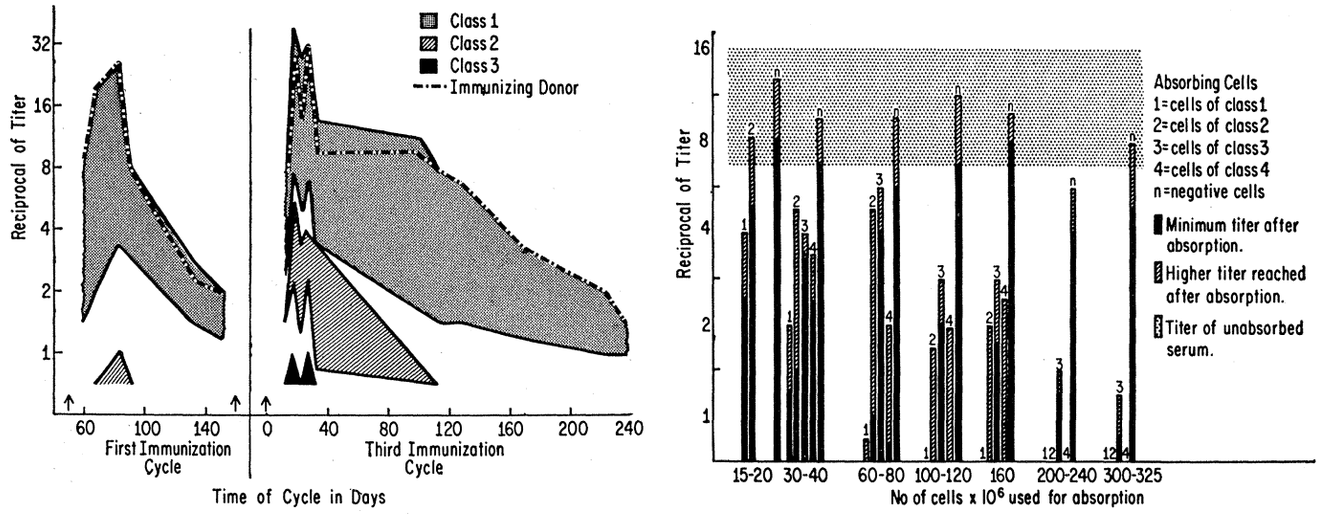

Fig. 1 (left). The range of titers obtained with serum obtained from subject BC at various times after immunization. The ranges cover replicate determinations with cells from seven subjects in class 1, five in class 2, and ten in class 3, together with single determinations from immunizing donor MF. Fig. 2 (right). Residual titers after absorption of undiluted serum are shown. Each bar represents the range of titers obtained in from two to six determinations; cells from two to four class 1 donors were used as indicator. Although complete absorption for class 1 cell was not obtained with undiluted serum, serum diluted 1:2 or more could be completely cleared.

varied from 17 to 58 percent with other antiserums to HL-A3 and from 19 to 72 percent with BC.

The strength of reaction obtained with cells from a given donor was constant; all reactions were checked at least twice and some ten times. Several families segregating for HL-A3 were tested, the children of a class 1 parent reacted as class 1, the children of a class 2 parent as class 2, and so on. The genotypically homozygous child of two heterozygous class 1 parents could not be distinguished from his parents or his heterozygous siblings by the strength of his reactions with the 12 samples of BC serum or with five other antiserums to HL-A3; gene dose effects were not obvious in this case.

The 19 serum samples (12 of BC, 5 of Storm, L'Heritier, and Thornton) were then subjected to absorption at dilutions ranging to 1 : 16, with buffy coat cells, the amount varying from 1.5×10^7 to 3×10^8 per milliliter. Absorptions were performed in two steps, each being 15 minutes at room temperature, with cells from each of the four classes and with cells from three HL-A3 negative donors, BC, EY, and GPK. Absorptions carried out with undiluted serum are summarized in Fig. 2. Class 1 cells readily absorbed even undiluted serum at concentrations of 10^8. Absorption by class 1 cells was complete for cells of all classes. At this concentration, class 2 cells would remove activity for other class 2 cells and for class 3, but frequently left some activity for class 1 cells (Fig. 2). Class 2 cells could remove all activity for class 1 cells at this cell concentration, provided that the serum was diluted 1:2 before absorption. Class 2 cells could completely absorb the undiluted serum when the concentration reached 2×10^8 cells. Complete absorption was not achieved with class 3 cells at the highest concentration tested (3.25×10^8 cell/ml) but was complete when the serum was diluted. Specificity controls, included at each serum dilution and cell concentration step, indicated no appreciable loss of activity. A second form of specificity control was also tested. Serum BC was mixed with a known antiserum to HL-A2 and absorbed with HL-A2–negative HL-A3–positive cells. Again there was no nonspecific absorption since the titer of antiserum to HL-A2 was unaffected. Cross-reactivity of the type reported by Svejgaard and Kissmeyer-Nielsen (*3*) appeared unlikely, since cells from each cell class could absorb for all other positive cells under appropriate conditions. Most significantly, class 3 cells could remove all activity from samples of BC, Storm, or Thornton even when the class 3 lymphocyte failed to give a positive reaction with that serum in the sensitive two-stage procedure.

These results affect both the interpretation of histocompatibility typing and the sublocus hypothesis (*6*). If an incompletely reacting antiserum to HL-A3 is used for donor-recipient typing, a class 3 or class 4 cell may be falsely classified as negative; thus, a graft from a class 1 donor to a class 3 recipient might be wrongly represented as a mismatch, and a graft from a class 3 donor to a negative recipient might be considered as a match. The "first sublocus" has been regarded as comprising HL-A1, -2, -3, and other mutually exclusive specificities (*6*). A few examples of cells carrying three specificities at this "sublocus" were previously known. The frequency with which HL-A3 is detected by absorption or through the use of highly reactive oligospecific serum BC suggests that many haplotypes determine more than one specificity of the LA series and that the determination of HL-A2 and HL-A3 cannot be at the same point locus. The ability of class 3 cells to absorb activity from all of the antiserum to HL-A3 tested indicates that the gene frequency of HL-A3 is higher than generally supposed. Over 5 percent of haplotypes have more than one of the first locus specificities. These data are consistent with an interpretation that the individual subloci, of which there are probably more than three, resemble the Rh locus. Thus, both positive and negative associations between specificities controlled by the same cistron or sublocus (*3*) are to be expected.

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Fungal Morphogenesis: Cell Wall Construction in Mucor rouxii

Abstract. *An autoradiographic study revealed two different patterns of cell wall construction associated with two types of morphogenesis in* Mucor rouxii. *In hyphae, the cell wall was preferentially synthesized in the apical region; these cylindrical walls seemed to be generated by a sharply descending gradient of wall synthesis radiating from the apex. In spherical cells (germinating spores, yeast cells), wall formation occurred largely, if not entirely, in uniformly dispersed fashion over the entire cell periphery.*

The vegetative morphology of *Mucor rouxii* is highly dependent on the environment. By suitable adjustment of the concentrations of carbon dioxide, oxygen, hexoses, and other factors (*1–3*), the fungus may be grown in a variety of forms ranging from slender hyphae to spherical budding yeast cells. This well-defined dimorphic capacity makes *M. rouxii* a convenient subject for exploring the molecular basis of vegetative morphogenesis in fungi.

Our previous studies on *M. rouxii*, when considered with the observations of others on the apical growth of fungi (*4, 5*), led to the hypothesis that fungal dimorphism might result from two different modes of cell wall construction: (i) apically localized in hyphae and (ii) uniformly dispersed in budding yeast cells (*6*). The present study was undertaken to test this proposal.

We obtained cylindrical and spherical cells by germinating spores of *M. rouxii*, IM-80, in yeast extract-peptone-glucose (YPG) medium (*1*)—hyphae were grown in medium containing 0.01 percent glucose and incubated under purified nitrogen; yeast cells were formed in medium with 0.1 percent glucose incubated under 30 percent CO_2 and 70 percent purified nitrogen (*3*). Prior to emergence of hyphae or yeast buds, spores undergo an obligatory phase of spherical growth (*7*); these spherical cells ("swollen" spores) were also examined.

To reveal the pattern of cell wall construction, we labeled growing cells with *N*-acetyl-D-[GL-^{3}H]glucosamine (*8*). Spores of *M. rouxii* (2×10^4 per milliliter) were inoculated in 20 ml of YPG medium as specified above, supplemented with 0.01 percent *N*-acetylglucosamine, and incubated in specially designed culture tubes (*3*). Cultures were chilled and centrifuged, and most of the supernatant fluid was discarded, leaving the cells suspended in about 2 ml. Suspensions of chilled unlabeled cells were flushed with the gas under which they were grown to eliminate any air introduced during centrifugation, and the temperature was again brought to 28°C. Cell suspensions were then added to 200 μc of tritiated *N*-acetylglucosamine and incubated anaerobically for 1 or 5 minutes. Cells were immediately killed by addition of an equal volume of 2*M* HCl. After being washed with water, the cells were extracted with 3 ml of alkaline ethanol (aqueous 1*M* NaOH : 95 percent ethanol, 1 : 2, by volume) in a boiling water bath for 5 minutes. This procedure removed all cytoplasmic radioactivity without destroying the original shape of the cell. The resulting cell ghosts were stained with congo red, heat-fixed onto a microscope slide, and coated with Kodak nuclear emulsion NTB-3 by the dipping method. Slides were exposed for several days and then developed. Silver grains on the autoradiograph correspond largely, if not entirely, to glucosamine molecules incorporated into two main cell wall polymers—chitin and chitosan (*9*).

The observed patterns of cell wall construction conformed to those previously postulated for cylindrical and spherical cells (Fig. 1). The hyphae exhibited a highly localized apical pattern of wall formation (Fig. 1E). Cell wall synthesis occurred mainly in the apical dome, an approximately hemispherical surface of about 4 to 6 μ in diameter. Rates of wall synthesis along the hyphae were measured by quantitative autoradiography (Fig. 2). A sharp gradient of cell wall synthesis exists in the apical region; the highest rate occurred at, or within 1 μ of, the zenith of the apical dome. Within 3 μ from

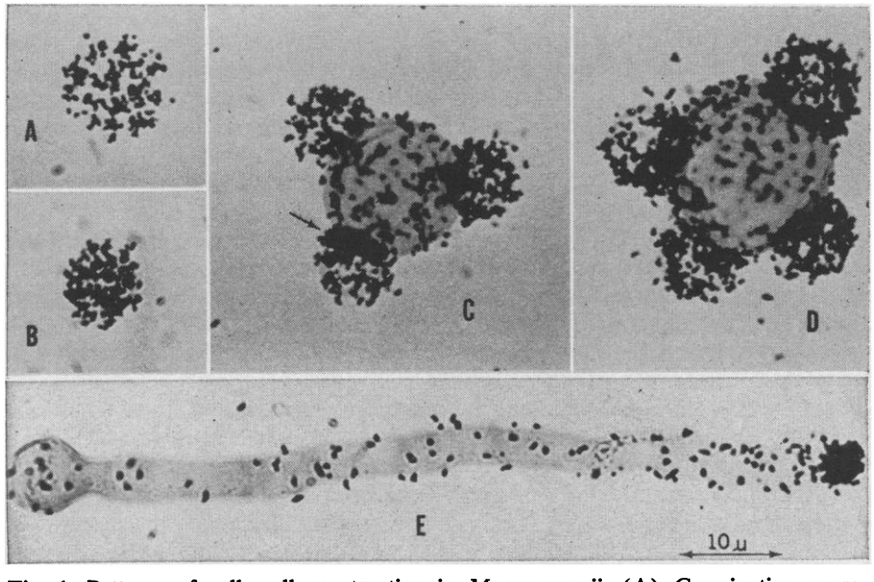

Fig. 1. Patterns of cell wall construction in *Mucor rouxii*. (A) Germinating spores prior to budding; (B) germinating spores prior to hyphal tube emergence; (C) and (D) yeast cells with three and four buds; and (E) hypha. Cells are 3 hours old in A and B and 12 hours old in C to E. All cells were exposed to tritiated *N*-acetyl-D-glucosamine for 5 minutes.