11. Nordlie, R. C. and Arion, W. J., J. Biol. Chem. **240**, 2155 (1965).
12. DeDuve, C. and Berthet, J., *in* "International Review of Cytology" (G. H. Bourne and J. F. Danielli, eds.), Vol. 3, p. 225. Academic Press, New York (1954).
13. Lee, Y. P. and Lardy, H. A., J. Biol. Chem. **240**, 1427 (1965).
14. Folch, J., Lees, M., and Stanley, G. H. S., J. Biol. Chem. **226**, 497 (1957).
15. Miller, J. E. and Cornatzer, W. E., Biochim. Biophys. Acta **125**, 534 (1966).
16. Fiske, C. H. and Subbarow, Y., J. Biol. Chem. **66**, 375 (1925).
17. Parker, F. and Peterson, N. F., J. Lipid Res. **6**, 455 (1965).
18. Brante, G., Nature **163**, 651 (1949).
19. Tsao, S. S. and Cornatzer, W. E., Lipids **2**, 41 (1967).
20. Hall, J. C., Sordahl, L. A., and Stefko, P. L., J. Biol. Chem. **235**, 1536 (1960).
21. Youngs, J. N. and Cornatzer, W. E., Proc. Soc. Exptl. Biol. Med. **112**, 308 (1963).
22. Youngs, J. N. and Cornatzer, W. E., Proc. Soc. Exptl. Biol. Med. **120**, 281 (1965).
23. Tata, J. R., Nature **213**, 566 (1967).
24. Garbus, J., DeLuca, H. F., Loomans, M. E., and Strong, F. M., J. Biol. Chem. **238**, 59 (1963).
25. Tsao, S. S. and Cornatzer, W. E., Lipids **2**, 424 (1967).

Received Jan. 20, 1969. P.S.E.B.M., 1969, Vol 131.

The "Thompson" Specificity and the HL-A Human Leukocyte System* (33906)

ROY L. WALFORD, GARY M. TROUP, EDITH ZELLER, EDWARD SHANBROM, AND ELAIN FEINGOLD

Department of Pathology, University of California School of Medicine, and Hyland Laboratories, Los Angeles, California 90033

Each gene or chromosomal region of the main human leukocyte antigenic system, HL-A, is manifested by multiple factors which segregate together as units in family studies (1, 2). Within the HL-A region, several subloci have been identified. The factors within each sublocus appear mutually exclusive to one another. For example, only one of the factors HL-A 1, 2, 3, Lc-11, and Dausset 15 and 17 of the first sublocus has been observed to be present on a single chromosome (2). Thus the factors behave as true alleles and no more than two have ever been observed phenotypically in the same person (2, 3).

In a prior immunogenetic analysis of the lymphocytes of a large family we detected crossover between a leukocyte specificity designated as "Thompson" and the known HL-A factors (4). We now report the finding of crossover in one of four additional families in which HL-A and "Thompson" segregation were followed. At the same time we have noted that when a population of unrelated donors was carefully and repeatedly typed for four mutually exclusive factors of the HL-A system, namely HL-A 1.0, 2.0, 3.0 and Lc-11, Thompson showed, with a very occasional exception, a mutually exclusive or allelic relationship to these four. Familial and population analyses thus appear paradoxical and together suggest the possibility of gene interaction or epistasis.

Materials and Methods. All typing was done with the Terasaki microcytotoxicity test (5) as modified by us (6). The "Thompson" antiserum was produced by intradermal injection of leukocytes from a donor into a recipient preselected on the basis of similarity to each other with existing antisera (4). Absorption of the "Thompson" antiserum with all of 30 positively reacting cell samples from different donors and of different races completely removed cytotoxic activity. The serum can therefore be considered as monospecific within the limits of the absorption

* Supported in part by NIH Research Grant AI-05403.

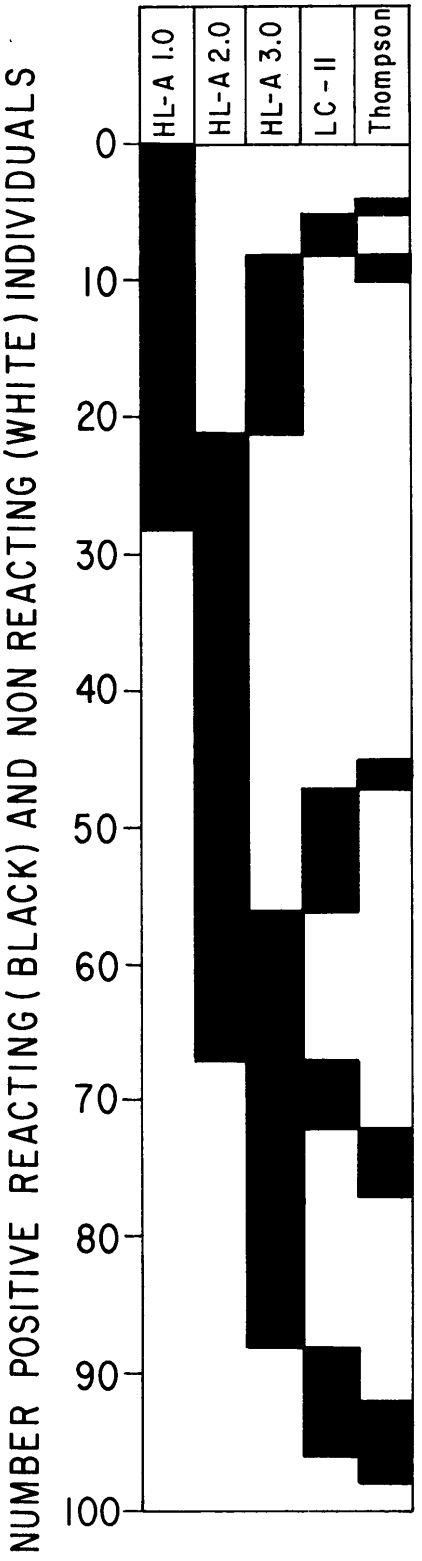

FIG. 1. Reactions of lymphocytes of 100 random donors with antisera to HL-A 1.0, 2.0, 3.0., Lc-11, and "Thompson" specificities: (black bars), positive reactions; (unmarked), negative. For example, 28 donors reacted with anti-HL-A 1.0; among this number 6 also reacted with anti-HL-A 2.0.

criterion (4). Absorption may not, however, distinguish between the few cross-reacting antibodies now known to exist within the HL-A system (2, 7).

Results. About 15% of Caucasians and over 30% of Negroes are "Thompson" - positive. Testing of Dausset's cell panel by one of us (RW) in Paris revealed that "Thompson" is distinct from Dausset factors 15 and 17. Typing of our 100-member Los Angeles cell panel (Fig. 1) showed that with two possible exceptions the "Thompson" specificity is absolutely exclusive to HL-A 1.0, 2.0, 3.0, and Lc-11. The two exceptions both involve weak positive reactions with anti-HL-A 3.0 sera in subjects whose cells are strongly reactive for both HL-A 1.0 and "Thompson". In studies now numbering up to 400 donors, the very occasional instance of "triplicate" positivity has always involved HL-A 3.0, "Thompson," and one of the other known specificities at the first sublocus. In addition, in these few "triplicate" instances the cells have always reacted only weakly with the HL-A 3.0 antisera. It is thus possible that the "Thompson" factor in a few individuals may show cross-reaction with anti-HL-A 3.0 sera, but that the genotypic exclusivity or allelism is absolute.

Despite the above relationships noted on a population basis, within one genetically informative family the "Thompson" factor showed evidence of segregation at least partially independent of the HL-A system (4). Furthermore, of two families in the Torino workshop for whom double backcross analysis could be accomplished, one showed crossover between Thompson and HL-A. Of four additional, informative families typed as part of the present study, one gave evidence of crossover. Immunogenetic analyses of two of these families, one (Levy) with and one (Walford) without evidence of crossover, are presented in Table I. If mother Levy is homozygous

TABLE I. Immunogenetic Analysis of Two Families Showing Double-Backcross Relation between the HL-A System and the "Thompson" Specificity.[a]

	Reaction with antisera detecting HL-A factors												
	HL-A					Lc-						HL-A	Reactions with
Family	1.0	2.0	3.0	7.0	8.0	11	18	20	Gallardo	Gallegos	Ortiz	chromosomes	Thompson antiserum
Levy													
Father	—	—	—	+	—	—	—	—	—	—	+	a/b	—
Mother	—	—	+	—	—	—	+	—	+	—	—	c/d	+
Child 1	—	—	+	+	—	—	+	—	+	—	+	a/c	+
2	—	—	—	+	—	—	+	—	—	—	+	a/d	+
3	—	—	+	+	—	—	+	—	+	—	+	a/c	—
4	—	—	+	+	—	—	+	—	+	—	+	a/c	+
5	—	—	—	+	—	—	+	—	—	—	—	b/d	+
6	—	—	—	+	—	—	+	—	—	—	+	a/d	+
Walford													
Father	+	+	—	—	+	—	+	+	—	—	—	a/b	—
Mother	+	—	—	—	+	—	—	—	—	—	—	c/d	+
Child 1	+	+	—	—	+	—	—	+	—	—	—	a/d	—
2	+	+	—	—	+	—	—	+	—	—	—	a/d	—
3	+	—	—	—	+	—	+	+	—	—	—	b/c	+

[a] Family Levy shows independent segregation, family Walford does not.

"Thompson"-positive, child no. 3 should give a positive reaction. If the mother is heterozygous, children nos. 1 and 4 reflect crossover with, or independence from the HL-A system.

Discussion. It is clear from our population results (Fig. 1) that the "Thompson" specificity is strongly related phenotypically and in an inverse, allelic manner to other factors of the first HL-A sublocus. The genotypic relationship suggested by the family studies are less clear. If crossover is indeed not infrequent, the combined observations of phenotypic exclusivity and partially independent segregation might suggest gene interaction, either between genes located at some distance apart on the same (HL-A) chromosome or separately present and at different loci on the paired (HL-A) chromosomes. For example, if a silent chromosome (Lc-X) of the HL-A system is only expressed when the Thompson gene is also present, one might explain the results of the family (Levy) by assuming the father to be homozygous Lc-X/Lc-X and the mother to be HL-A 3.0/Lc-X, with the Thompson gene being on the Lc-X-carrying chromosome of the mother but not at the same locus as Lc-X. Then children nos. 1 and 4 must represent cross-over, with interaction of genes on paired chromosomes to yield the final antigenic factor reactive with the "Thompson" antiserum. We do not stress this precise interpretation (which would predict finding Thompson-positive children born to phenotypically-negative parents) because further genetic studies may suggest others.

Summary. Population and family studies of the Thompson specificity and four factors of the first sublocus of the HL-A main human leukocyte system are described. The Thompson specificity is strongly allelic or pseudoallelic to the other factors of the sublocus. At the same time, evidence of crossover between Thompson and HL-A has been found in 3 of 7 families studied to date. Taken together, population and family data suggest the possibility that the Thompson factor is the expression of gene interaction or epistasis involving the HL-A and some other system.

1. Ceppellini, R., *in* "Advances in Transplantation" (J. Dausset, J. Hamburger, and G. Mathe, eds.), p. 195. Munksgaard, Copenhagen (1968).

2. Dausset, J., Walford, R. L., Colombani, J.,

Legrand, L., Feingold, N., and Rapaport, F., Proc. Intern. Congr. Transpl. Soc., 2nd, in press.

3. Walford, R. L., Wallace, O., Shanbrom, E., and Troup, G. M., Vox Sanguinis **15**, 338 (1968).

4. Walford, R. L., Shanbrom, E., Troup, G. M., Zeller, E., and Ackerman, B., *in* "Histocompatibility Testing 1967" (E. S. Curtoni, P. L. Mattiuz, and R. M. Tosi, eds.), p. 221. Munksgaard, Copenhagen (1967).

5. Terasaki, P. S. and McClelland, J. D., Nature **204**, 998 (1964).

6. Troup, G. M. and Walford, R. L., Am. J. Clin. Pathol., in press.

7. Kissmeyer-Nielsen, F., Svejgaard, A., and Hauge, M., Nature **219**, 1116 (1968).

Received Jan. 21, 1969. P.S.E.B.M., 1969, Vol. 131.

Distribution and Degradation of Sublethal Doses of I^{125} Labeled Endotoxin from *Salmonella enteritidis* in Mice* (33907)

JIBAN D. GUPTA[1] AND CHARLES E. REED[2]
(Introduced by Charles V. Seastone)
Department of Medicine, University of Wisconsin Medical School, Madison, Wisconsin 53706

Injection of minute quantities of lipopolysaccharide extracted from cell walls of gram-negative organisms (endotoxin) produces a variety of physiological responses of several target organs. Information about the distribution of endotoxin in the target organs would be helpful in elucidating the mechanism of those effects, and radioactive labeled endotoxins have been employed for this purpose.

Braude and co-workers (1, 2) studied the distribution of lethal doses of ^{51}Cr labeled endotoxin from *E. coli* injected intravenously in rabbits and mice, and attempted to correlate the physiological response with the distribution of radioactivity. Similarly, Starzecki *et al.* (3) studied the plasma clearance and tissue distribution of ^{51}Cr labeled endotoxin in normal and endotoxin-resistant dogs. Other workers (4–6) also studied the distribution of endotoxins from other bacterial species labeled with other isotopes. However, in these studies a lethal dose of endotoxin had to be injected because of the low specific activity of the endotoxin. High specific activity can be obtained by labeling purified endotoxin from *Salmonella enteritidis* with ^{125}I (7). We have investigated the possibility that this preparation might be useful in studying the distribution of small nontoxic amounts of endotoxin, but serum enzymes degrade the endotoxin molecule *in vitro*, removing a large part of the radioiodinated lipid from the bulk of the lipopolysaccharide (8). If similar degradation occurs *in vivo*, interpretation of the results of tracer injection studies would become complex, for part of the radioactivity would be iodinated fatty acids or free iodide. The present experiments on the distribution of radioactivity after injection of ^{125}I labeled endotoxin in mice showed that degradation of the endotoxin complex does indeed occur *in vivo* and the radioactivity in some tissues is due more to iodine freed from the endotoxin than to the localization of the injected endotoxin complex itself. By comparing the distribution of radioactivity after injection of chromium and iodine labeled endotoxin some information can be obtained about the distribution of the different parts of the endotoxin complex.

Methods. Experimental animals. Swiss Webster mice of either sex weighing 20–25 g were housed in cages containing 4–5 animals, given food and water *ad libitum* and kept for 2 hr at 25 or 37° before the intravenous injection of endotoxin and held at the same temperature for the duration of the experiment. At various times after injection the animals were anesthetized with ether, decapitated and the blood was collected directly

* Supported by USPHS Grant no. A1 04412, HE 015626 and OH 00044.

[1] Present address: Childrens Medical Research Foundation, Royal Alexanda Hospital for Children, Camperdown, N.S.W. 2050 Australia.

[2] Reprint requests to Dr. Reed.