Possible Family Relationships Revealed by Determination of HLA-transplantation Antigens

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A determination of the transplantation antigens belonging to the HLA-A and HLA-B series was carried out with muscle and skin tissue by means of a microabsorption method. The results indicate that two families have been buried – one in each of the two tombs. No conclusion could be reached on the possibility of an admixture of Caucasian genes.

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The genes of the human histocompatibility system, the HLA system, are situated on the short arm of chromosome No. 6, (Fig. 1). The HLA system is a subject of intense research, because of its overall importance for donor-recipient matching in allotransplantation, and because of the role of the HLA antigens in general immunological recognition. Furthermore, the great polymorphism of the HLA system, and the fact that the antigens are expressed with variable frequencies in different ethnic groups, also makes the system a useful tool in ethnographic studies. (Bauer & Danilovs 1980).

The HLA antigens are structures of glycoproteins with great resemblances to the structure of the gammaglobulins, and they are found on the membranes of most nucleated cells in the body. An HLA determination is usually carried out with live lymphocytes, and the antigens of the HLA-A,-B,-C, and -DR series are all serologically recognizable, the HLA-A,B,C antigens on all lymphocytes, the HLA-DR antigens on B-lymphocytes. (Reviews: Bodmer 1978; Kissmeyer-Nielsen 1981).

The HLA determination of the mummies from Qilakitsoq was attempted because the bodies had been preserved under fairly favourable conditions: they were in fact freeze-dried by nature, and thus the structure of the HLA antigens could be expected to survive inside the larger muscles and in epidermis.

The purpose of the study was mainly to reveal possible family relations, and then to find whether the HLA antigens expressed in those Eskimos were typical of living Eskimo populations. The study involved the antigens of the HLA-A and HLA-B series, and the analyses performed by a microabsorption technique developed in this laboratory for identification purposes (Hansen & Gürtler 1979, 1982).

Below a short description is given of the technique used, with examples of the absorption diagrams, and the combined results of the analyses. Detailed descriptions of the investigations have been published elsewhere (Hansen & Gürtler 1983).

Technique

About fifty different HLA-antisera of well defined specificity and antibody titre were chosen for the experiments. For each absorption aliquots of 150–200 microlitres of centrifuged tissue, previously minced and softened with a balanced salt solution, were mixed with 50 microlitres of specific HLA antiserum. After incubation for one hour at 37°C, the serum was recovered by centrifugation. Titrations of absorbed and unabsorbed se-

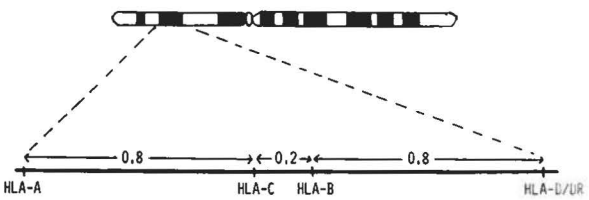

Fig. 1. Chromosome map of the HLA system, 1980.
Here the HLA-A series comprises 17 alleles, HLA-A1, A2, A3, etc., the HLA-B series 33 alleles, HLA-B5, B7, B8 etc. The HLA-C series 8 alleles, HLA-Cw1, Cw2, Cw3 etc. The HLA-D/DR series comprises about 10 defined alleles.
The map distance is given in centimorgans, e.g. a distance of 0.8 cM between the HLA-A and HLA-C locus means that the frequency of recombination between these two loci is 0.8 %. (WHO & IUIS Nomenclature Committee 1980).

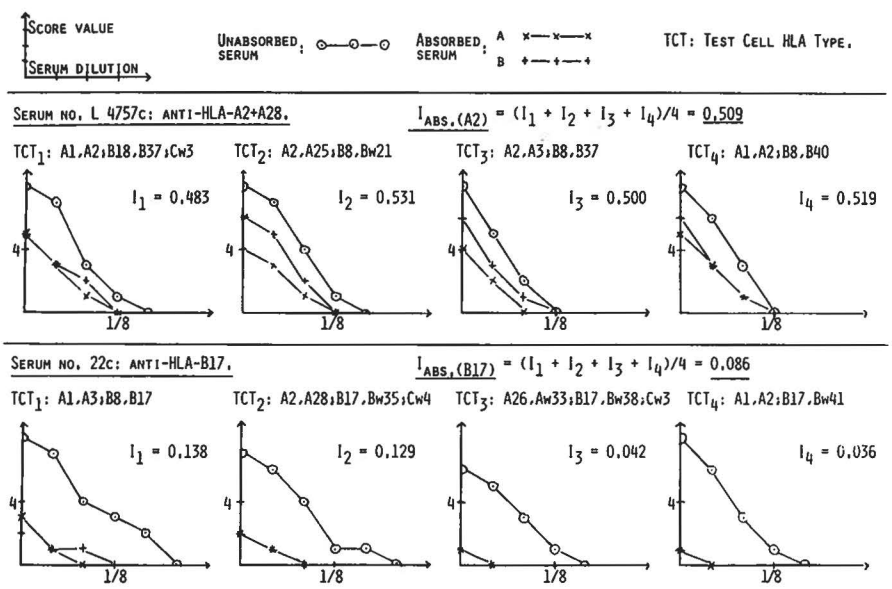

Fig. 2 Examples of specific absorption (serum No. 22c) and non-absorption (serum No. L 4757c) from the HLA typing of mummy No. I/4. Reduction of antibody activity in the two reagents is measured by means of test cells from different donors with the appropiate HLA types, and unabsorbed and absorbed sera are tested in the dilutions Undiluted, ½, ¼, ⅛, 1/16, 1/32. It can be seen that serum No. 22c has almost completely lost the ability to kill HLA-B17-positive test cells. The proportion of dead test cells (%) is counted according to the score values (sc):0–5%: sc=0, 6–15%: sc=1, 16–30%: sc=2, 31–45%: sc=3, 46–55%: sc=4, 56–70%: sc=5, 71–85%: sc=6, 86–95%: sc=7, 96–100%: sc=8. The absorption index, I, was estimated twice for each test cell donor, and was calculated as:

$$I = \frac{\text{area below graph for one absorbed serum sample}}{\text{area below graph for unabsorbed serum sample}}$$

The final index for the reduction of antibody activity in one serum is the average value of the indices obtained by the testing of the duplicate absorptions with the different test cell suspensions. The final Absorption Indes, $I_{abs.}$, for one HLA antigen is obtained as the average value of the indices for two or more antisera defining that antigen.
From Hansen, H. E. & Gürtler, H. 1983. HLA types of mummified Eskimo bodies from the 15th century. Amer. J. Phys. Anthropol. 61: 447–452.

rum were made in Hamax microchambers, and the ability of mummified tissue to remove specific HLA antibody from a given serum was measured by means of the NIH-microcytotoxicity test (Bodmer 1978). Thus, when an antiserum, compared with the unabsorbed serum, had lost the ability to kill specificalty lymphocytes from a donor of the appropriate HLA type, the HLA antibody was retained in the tissue because that mummy was likely to possess the corresponding antigen. Examples are given in Fig. 2.

The reduction of antibody activity in an absorbed serum sample, compared with the antibody activity in the unabsorbed serum, is measured quantitatively by means of the Absorption Index (Hansen & Gürtler 1982), (Fig. 2). The absorption indices from all the antisera used are expressed in one diagram for each mummy. An example is given in Fig. 3.

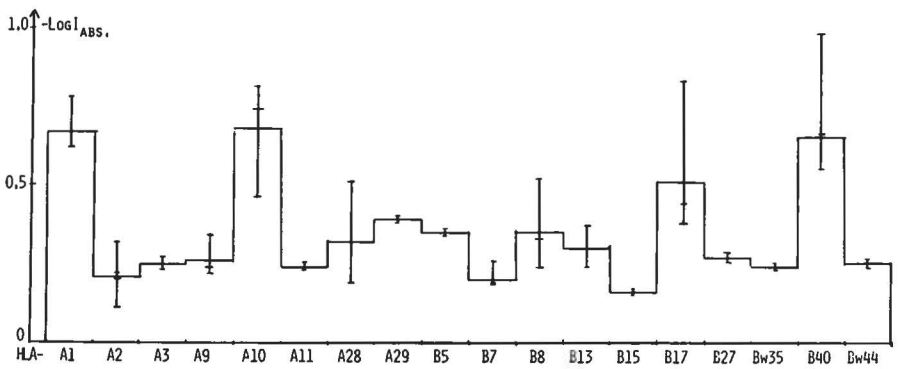

Fig. 3. Results of the HLA typing of mummy No. I/5. In the diagram the HLA antigens investigated for all the mummies are listed along the X-axis. Along the Y-axis is marked the negative logarithm of the absorption indices for each of the antigens. The use of the negative logarithm makes the HLA types directly evident as the highest columns in the diagram. For each antigen the horizontal line is the negative logarithm of the average value of two or more indices, each of which is shown on the vertical line, that is one value for each of the different HLA antisera defining that antigen.
The HLA types of No. I/5 are: HLA-A1,A10;B17,B40. From Hansen, H. E. & Gürtler, H. 1983. HLA types of mummified Eskimo bodies from the 15th century. – Amer. J. Phys Anthropol. 61: 447–452.

Results and discussion

The tests were done on six adult women and the 4-5-year-old boy. The baby was excluded because of its size, as tissue could not be obtained without molestation. The results of the HLA-determinations were correlated with the placing in the two tombs and with the age at death of each person, as reported by the Danish National Museum (Fig. 4).

The results indicate that one family was buried in each tomb. A possible interpretation of the HLA results is: Tomb I: a grandmother (I/5), two daughters (I/4 & I/3) and one (two) grandchild(ren); Tomb II: two sisters (II/6 & II/8) and a young girl (II/7) who may have been the daughter of either of them. Theoretically the three eldest women, I/5, II/6 and II/8, could be sibs, in which case their parents had the HLA types A1,B17//A28,B5 and A10,B40//A28,B40 respectively, but this cannot be proved. II/6 may have been the mother of I/4 and I/3, but the young woman, II/7, cannot have been the daughter of any of the women in Tomb I, nor can she have been the mother of I/2.

A total of eight different HLA antigens were observed among the seven mummies. HLA-A9, A28, B5, and B40 are frequent among present-day Eskimos, while HLA-A1, A10, B8 and B17 are rare, although they have been observed (Kissmeyer-Nielsen *et al.* 1971; McAlpine *et al.* 1974). The studies of HLA gene frequencies among living Eskimos involve comparatively small population groups, so when a system as polymorph as the HLA system is considered, poor information is obtained about the frequencies of rare alleles. Rare genes may accumulate in isolates and in family clusters, and the HLA genes represented in two random families are due to chance. Thus the presence of four HLA alleles, rare among Eskimos but more frequent among Caucasians (Bauer & Danilova 1980), including Danes (Hansen *et al.* 1979), cannot be considered to indicate Caucasion admixture. These genes might just as well represent the genuine Inuit gene pool around AD 1475.

Age at the time of death	No.	Sex	HLA-type
Tomb I:			
40-50 years	I/5	F	A1,A10;B17,B40
About 30 years	I/4	F	A9,A10;B17,B40
20-25 years	I/3	F	A9,A10;B8,B40
4-5 years	I/2	M	A9,A10;B8,B40
Tomb II:			
About 50 years	II/6	F	A10,A28;B5,B40
40-50 Years	II/8	F	A28;B5,B40
About 18 years	II/7	F	A28;B5

Fig. 4. The results of the HLA determinations of the seven mummies from Qilakitsoq.
The HLA types are correlated with information from the Danish National Museum on the placing in the two graves, and on the age at death of each person. F = female, M = male.

Acknowledgements

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