exercise rise in body temperature after swimming than after running or cycling may warrant further investigation.

The mechanism of exercise-induced asthma remains obscure. Hypocapnia (Fisher *et al.*, 1970), release of a humoral substance (McNeil *et al.*, 1966), metabolic acidosis providing release of a bronchoconstrictor agent (Seaton *et al.*, 1969), lactic acidemia causing increased ventilation and hypocapnia (Ward *et al.*, 1969), and hyperventilation reflexly causing bronchoconstriction (Crompton, 1968) have been mentioned by recent authors. It has been suggested (Rebuck and Read, 1968) that the condition may not be a single homogenous entity, and the variable protective effect of anticholinergic and bronchodilator agents tends to confirm this theory. Nevertheless, the highly specific nature of the response, its onset and abatement, its acceptance as a provocative test of labile bronchi in latent asthma (Jones, 1966), and the frequency and consistency with which it can be provoked imply that it is the consequence of specific aetiological factors. Certainly we must agree with Rebuck and Read (1969) that continuous measurement of blood gas tensions, blood analysis, and lung volumes during and after exercise is perhaps the most promising method of solving this fascinating problem.

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References

- American Academy of Pediatrics (1970). *Pediatrics*, **45**, 150.
 American Thoracic Society (1962). *American Review of Respiratory Diseases*, **85**, 763.
 Astrand, P. O., and Christensen, E. H. (1964). In *Aerobic Work Capacity in Oxygen and the Animal Organism*, ed. F. Dickens, E. Neil, and W. Widdas. New York, Pergamon Press.
 Blackburn, H., *et al.* (1967). In *Physical Activity and the Heart*, ed. M. J. Karvonen, and A. J. Barry. Springfield, Thomas.
 Crompton, G. K. (1968). *Thorax*, **23**, 165.
 Fisher, H. K., Holton, P., Buxton, R. St. J., and Nadel, J. A. (1970). *American Review of Respiratory Diseases*, **101**, 885.
 Jones, R. H. T., and Jones, R. S. (1966). *British Medical Journal*, **2**, 976.
 Jones, R. S. (1966). *British Medical Journal*, **2**, 972.
 Jones, R. S., Buxton, M. H., and Wharton, M. J. (1962). *British Journal of Diseases of the Chest*, **56**, 78.
 Jones, R. S., Wharton, M. J., and Buxton, M. H. (1963). *Archives of Disease in Childhood*, **38**, 539.
 Kish, L. (1965). *Survey Sampling*, p. 19. Chichester, Wiley.
 Kjellman, B. (1969). *Scandinavian Journal of Respiratory Diseases*, **50**, 41.
 Lindquist, E. F. (1953). *Design and Analysis of Experiments in Psychology and Education*, p. 237. Boston, Houghton Mifflin.
 McNeill, R. S., Nairn, J. R., Millar, J. S., and Ingram, C. G. (1966). *Quarterly Journal of Medicine*, **35**, 55.
 Pierson, W. E., Bierman, C. W., and Stamm, S. J. (1969). *Journal of Allergy*, **43**, 136.
 Poppius, H., Muittari, A., Kreis, K.-E., Korhonen, O., and Viljanen, A. (1970). *British Medical Journal*, **4**, 337.
 Rebuck, A. S., and Read, J. (1968). *Lancet*, **2**, 429.
 Seaton, A., Davies, G., Gaziano, D., and Hughes, R. O. (1969). *British Medical Journal*, **3**, 556.
 Sly, R. M. (1970). *Annals of Allergy*, **28**, 1.
 Ward, F. G., Gomes, S., and McNeill, R. S. (1969). *British Medical Journal*, **3**, 176.

Response of HL-A Identical Unrelated Individuals in the Mixed Lymphocyte Culture Test

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Summary

The immunological responsiveness as measured in the mixed lymphocyte culture test has been studied in 13 pairs of HL-A identical unrelated individuals. In all combinations stimulation occurred and it was frequently of a similar magnitude to that observed against non-HL-A identical subjects.

It is postulated that another locus, adjacent to the HL-A locus, is also responsible for the non-stimulation observed between HL-A identical siblings, and that observations made in the related situation should not be transposed to the unrelated situation without some reservation.

Introduction

The pre-eminence of the HL-A system in histocompatibility is now widely accepted and its importance is based on the

results of studies carried out in families. It has been shown that cells from HL-A identical siblings do not react in mixed lymphocyte culture (Amos and Bach, 1968; Bach, 1970) and that renal allografts exchanged between HL-A identical siblings are almost invariably successful (Hors *et al.*, 1970). On the basis of these observations the HL-A system has been adopted as the system on which selection of donors for cadaveric renal transplantation programmes is based. However, very little is known about histoidentity as defined by the HL-A system in the unrelated situation (van Rood and Eijssvoegel, 1970) and this work was undertaken in order to study this aspect, using the mixed lymphocyte culture test to measure true immunological identity between unrelated subjects.

Subjects and Methods

Lymphocytes from normal healthy staff members and from prospective renal allograft recipients were typed for their HL-A antigens by the lymphocyte cytotoxicity test. Computer analyses of the frequencies and associations of the antigens as determined in this laboratory have been carried out (Sharp *et al.*, 1970, 1971). Subjects in whom all four HL-A antigens could be unequivocally identified and whose phenotypes occurred more than once were selected for study in the mixed lymphocyte culture test. The HL-A phenotypes of the 17 subjects in the study are listed in Table I.

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PREPARATION OF CELLS

Cells for the mixed lymphocyte culture test were prepared from 100 ml of freshly drawn heparinized blood (20 IU heparin per ml whole blood). Twenty millilitres of blood was removed to narrow conical tubes and allowed to sediment at 37°C for one hour. The supernatants were then removed, centrifuged at 150 *g* for 10 minutes, and the cell buttons retained. The remaining blood was centrifuged over a mixture of Ficoll (7% w/v) and Hypaque (diatrizoic acid) (10% w/v), density 1.078, at 1,000 *g* for 20 minutes. The leucocyte bands were removed, washed once with Hanks's balanced salts solution (150 *g* for 10 minutes), and then divided into two parts. For the preparation of stimulating cells, mitomycin C (Kyowa) (30 µg/ml) was added to the suspension, which was incubated at 37°C for 20 minutes. The cells were then washed twice with Hanks's solution and finally suspended for counting in tissue culture medium. For the responding cells the leucocytes from the Ficoll-Hypaque and the sedimentation separations were pooled, washed twice with Hanks's solution, and finally resuspended in tissue culture medium. After counting, the concentrations of both cell suspensions were adjusted to 1.5×10^6 lymphocytes per ml.

This separation procedure usually gave a yield of 80-100 × 10⁶ lymphocytes from 100 ml of blood. Preparations of stimulating cells contained less than 5% polymorphs while the preparation of responding cells contained 20-30% polymorphs. Both preparations were essentially free of platelets.

CULTURE CONDITIONS

Cells were cultured at 37°C in 199/Earle's salt medium (Flow Laboratories) buffered with Hepes (Flow Laboratories) and supplemented with penicillin (120 U/ml), streptomycin (120 U/ml), and 25% AB pooled serum. Cultures were prepared in triplicate in plastic tubes and consisted of 1-ml volumes each of stimulating and responding cells.

Each experiment consisted of an HL-A identical, unrelated pair put up against each other in one-way culture both as stimulating and as responding cells. In addition, at least one non-HL-A identical subject was included as a stimulating and as a responding cell against each member of the pair.

In one experiment the response of an individual was measured against her HL-A identical sibling, as well as against HL-A identical and non-HL-A identical unrelated individuals.

Transformation was measured in 18 HL-A identical and in 33 non-HL-A identical unrelated situations.

On the sixth day 0.2 µCi of tritiated thymidine (specific activity 26 Ci/mole, Amersham) was added to each culture. After 18 hours the cultures were chilled to 0°C and harvested on to glass fibre filter pads (Whatman GF/C). Material remaining in the culture tube was washed on to the pad with 40 ml of ice-cold saline and acid-insoluble material precipitated on the filter with 20 ml of ice-cold 5% trichloroacetic acid. The filter pads were finally washed with 20 ml of methanol, placed precipitate side up in scintillation phials, and dried at 90°C for 48 hours, after which 10 ml of scintillation fluid was added. The samples were counted in a Packard scintillation counter.

ANALYSIS

The viability of responding cells and the non-responsiveness of stimulating cells were checked by incubating each type alone with phytohaemagglutinin (Wellcome) (0.02 ml per culture).

Results are expressed as the transformation index—that is, the ratio of the radioactive thymidine incorporation in the mixed culture to the radioactive thymidine incorporation in the negative control culture consisting of the subject's responding and stimulating cells. The negative control culture thus has a transformation index of 1.0.

Results

HL-A phenotypes of the 17 subjects in the study are listed in Table I. Subject 5 had no HL-A identical partner and was included only as a stimulating cell. Subject 6 was an HL-A identical sibling to Subject 1 and was included only as a stimulating cell. Subjects 8 and 13 were included only as stimulating cells because of low lymphocyte yields. For the same reason the five HL-A identical subjects (Nos. 9, 10, 11, 12, and 13) could not be tested in all combinations.

In Fig. 1 the results of four experiments are expressed diagrammatically. Every subject has responded to his HL-A

TABLE I—HL-A Phenotypes of Subjects

Subject No.	HL-A Phenotype				
1	1,	3,	7,	8,	4b
2	1,	3,	7,	8,	4b
3	1,	3,	MaKi(W14),	Te57(W17),	4a,
4	1,	3,	MaKi(W14),	Te57(W17),	4a,
5	2,	10,	FJH,		4a,
6*	1,	3,	7,	8,	4b
7	1,	2,	8,	Te57(W17),	4a,
8	1,	2,	8,	Te57(W17),	4a,
9	1,	2,	8,	12,	4a,
10	1,	2,	8,	12,	4a,
11	1,	2,	8,	12,	4a,
12	1,	2,	8,	12,	4a,
13	1,	2,	8,	12,	4a,
14	1,	3,	8,	12,	4a,
15	1,	3,	8,	12,	4a,
16	1,	2,	5,	8,	4a,
17	1,	2,	5,	8,	4a,

*Sibling to Subject 1.

FIG. 1—Transformation indices of four subjects with various stimulating cells.

TABLE II—Transformation Indices between HL-A Identical Subjects

Experiment No.	Responding Cell	Stimulating Cell	Transformation Index
1	1	2	4.5
2	2	1	11.9
3	3	4	21.2
4	4	3	5.4
5	7	8	3.5
6	9	10	2.5
7	9	13	7.4
8	10	9	1.8
9	10	11	12.6
10	11	9	6.1
11	11	12	23.2
12	11	13	7.7
13	12	10	18.7
14	12	13	22.6
15	14	15	7.5
16	15	14	21.0
17	16	17	29.6
18	17	16	6.8

identical partner. Subject 1 has shown no stimulation (transformation index 0.8) against her HL-A identical sibling (Subject 6) but has responded well to the HL-A identical unrelated subject. In only 8 of the 18 experiments was stimulation against the HL-A identical partner less than against all the non-identical partners.

The transformation indices between all the combinations of HL-A identical subjects tested are shown in Table II. The results of the whole series of experiments are expressed in another way in Fig. 2. There is no significant difference between

FIG. 2—Transformation indices of HL-A identical and non-HL-A identical unrelated subjects.

the mean transformation index of the HL-A identical pairs (11.9 ± 8.6) and the non-HL-A identical pairs (10.1 ± 3.8), though the scatter in the HL-A identical group is much greater.

Discussion

If the HL-A antigens are solely responsible for reactivity in the mixed lymphocyte culture test then stimulation should not occur in tests between HL-A identical individuals. That this is so in HL-A identical siblings has been unequivocally established.

Our results show that unrelated individuals identical for four HL-A antigens can stimulate in the mixed lymphocyte culture test. Since this study was begun similar results have been reported by several other groups. Kissmeyer-Nielsen *et al.* (1970) studied four HL-A identical unrelated pairs and found none of them to be non-stimulatory. Eijssvoegel *et al.* (1971) studied six unrelated individuals, all of whom carried the antigens HL-A1, 3, 7, and 8, and found three of these to be reciprocally non-stimulatory. These three pairs were the only ones in the overall series of 26 HL-A identical unrelated pairs of individuals which did not stimulate. In their series of four HL-A identical unrelated individuals, all carrying the antigens HL-A1, 2, 8, and 12, Bach *et al.* (1971) found one pair to be reciprocally non-stimulatory but found all the other 10 combinations of HL-A identical pairs to be stimulatory to the same extent as against non-identical individuals.

The ability of unrelated individuals identical for the first and second segregant series of HL-A to stimulate in the mixed lymphocyte culture test has, therefore, now been established. It also seems that in such combinations stimulation is more likely to occur than non-stimulation, and that the levels of

transformation achieved are comparable with those achieved by non-HL-A identical pairs.

Some valid explanation must be found for this apparent discrepancy between the related and unrelated situation in which the pairs are identical for all four HL-A antigens. Heterogeneity of the HL-A antigens has been emphasized (Thorsby *et al.*, 1971a), and this must be considered as a possible explanation. However, most of the individuals in this series carry the extremely well-defined antigens HL-A1, 2, 3, 7, 8, and 12, none of which (with perhaps the exception of HL-A12) have shown any indication of heterogeneity (Fourth International Histocompatibility Workshop, 1970). Splitting of antigens much less well defined than these is an accepted development of our increasing knowledge of the HL-A system (Thorsby *et al.*, 1971b), but if even well-defined antigens are found to be heterogeneous, our concepts of "HL-A identity" must be widened to encompass a system which is incredibly complex. If only the minority of HL-A identical unrelated pairs stimulated it could be accepted that the antigens were, in these cases, heterogeneous. However, stimulation seems to be the rule, and only occasional pairs fail to stimulate.

In the absence of extensive family studies it is not possible to say that two unrelated individuals are genotypically as well as phenotypically identical. However, some haplotypes are extremely common in the Caucasian population—HL-A1 and HL-A8, HL-A2 and HL-A12, HL-A3 and HL-A7 (Sharp *et al.*, 1970; Fourth International Histocompatibility Workshop, 1970)—and we have recently observed strong positive associations between the antigens HL-A1 and Te57(W17), HL-A3 and MaKi(W14) (Sharp *et al.*, 1971). Since most of the individuals in our study possessed only antigens which have been shown to be positively associated, we feel that at least some of the pairs are likely to have been genotypically identical. It has already been shown that some pairs genotypically identical for the four HL-A antigens are stimulatory (Kissmeyer-Nielsen *et al.*, 1970).

Previous exposure of the subjects to non-HL-A antigens and subsequent sensitization also seems unlikely as a cause for stimulation as more than half of our subjects have never been exposed to foreign tissue antigens either by blood transfusion or by pregnancy.

We feel then that these results can only be satisfactorily explained if there is another factor in addition to the antigens of the first and second series of the HL-A locus operative in the mixed lymphocyte culture reaction and presumably in organ transplantation. This factor, however, must be situated very close to the HL-A locus as the HL-A identical sibling data indicate that crossing over between the HL-A locus and the "mixed lymphocyte culture locus" does not occur to any significant extent.

It is likely that the observations from the mixed lymphocyte culture test can be extended to organ transplantation. That there is minimal rejection of kidneys transplanted between HL-A identical siblings is well established, but few transplants have been carried out with HL-A identical unrelated donors. In the period from September 1967 to July 1971 356 grafts were carried out in Sydney with kidneys from cadaver donors. In four of these operations donor and recipient were shown to be HL-A identical. Three pairs carried the antigens HL-A1, 2, 8, and 12 while the fourth carried the antigens HL-A1, 10, Te57(W17), and S Ly3. Two of these patients maintained excellent function at 21 and 12 months respectively after operation. In the third patient, for whom this was a second graft, rejection was controlled with difficulty for seven months after which it became uncontrollable and the graft was removed. The fourth patient (HL-A1, 10, Te57(W17), and S Ly3), at only three months postoperation, has suffered two acute rejection episodes, at two and five weeks, both of which were controlled with increased immunosuppression. In only the last case was the mixed lymphocyte culture test carried out, and low-grade but definite stimulation was observed (transformation index 4.5).

The results observed in mixed lymphocyte culture tests and

kidney transplantation between HL-A identical unrelated individuals and the difficulty of establishing a statistically significant correlation between HL-A matching grade and clinical outcome of the graft (Stewart *et al.*, 1970) indicate that the observations in siblings cannot be directly transposed to the unrelated situation. If the HL-A locus, as currently defined by two segregant series, is not solely responsible for mixed lymphocyte culture reactivity the additional factors responsible for this reactivity may also contribute to histocompatibility and be relevant to transplantation. Elucidation of these factors could help us to define histoidentity more precisely and then the results of transplantation with unrelated cadaveric donors may more closely approach the excellent results of HL-A identical sibling transplants.

References

- Amos, D. B., and Bach, F. H. (1968). *Journal of Experimental Medicine*, **128**, 623.
 Bach, F. H. (1970). *Science*, **168**, 1170.
 Bach, F. H., *et al.* (1971). *Tissue Antigens*, **1**, 39.
 Eijssvoogel, V. P., *et al.* (1971). *Transplantation Proceedings*. In press.
 Fourth International Histocompatibility Workshop (1970). *Proceedings*, Copenhagen, Munksgaard.
 Kissmeyer-Nielsen, F., Sveigaard, A., Sorensen, S. F., Nielsen, L. S., and Thorsby, E. (1970). *Nature*, **228**, 63.
 Hors, J., Feingold, N., Fradelizi, D., and Dausset, J. (1970). *Lancet*, **1**, 609.
 Sharp, A. M., Cook, K. M., and Johnston, J. M. (1970). *Nature*, **227**, 950.
 Sharp, A. M., Bashir, H. V., Johnston, J. M., and Cook, K. M. (1971). In press.
 Stewart, J. H., *et al.* (1970). *British Medical Journal*, **3**, 487.
 Thorsby, E., *et al.* (1971a). *Tissue Antigens*, **1**, 32.
 Thorsby, E., Lindholm, A., Sandberg, L., and Staub-Nielsen, L. (1971b). *Vox Sanguinis*, In press.
 van Rood, J. J., and Eijssvoogel, V. P. (1970). *Lancet*, **1**, 698.

Transience of Immune Responses to Tumour Antigens in Man

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Summary

By examining serial serum samples from patients undergoing surgical removal of malignant tumours an increased incidence of tumour-specific antibodies has been detected. Antibody responses to tumour neoantigens seem to be transient, and this is held to account for the rarity of detections where a single sample of serum is examined.

Introduction

A large body of opinion holds that human tumours, like experimental animal tumours, possess tumour-specific antigens (tumour neoantigens) (Fairley, 1969, 1970; Mathé, 1969, 1970; Alexander, 1970; Klein, 1970; Woodruff, 1970). Yet only in a minority of cancer patients have antibody responses to tumour neoantigens been detected (Graham and Graham, 1955; Wilson *et al.*, 1963; Southam, 1965; Dore *et al.*, 1967; Lewis, 1967; Lewis *et al.*, 1969).

In a previous communication from this department, Hodgkinson and Taylor (1969) reported the detection of tumour-specific antibodies in the sera of only 2 out of 34 cases of human carcinoma. One reason suggested for the low frequency of tumour antibody detection was the possibility that while the tumour is in situ a slow constant release of tumour antigen combines with any antibody produced so that the latter is not detectable in the serum. The work reported here is an investigation of this possibility, and the findings suggest that reaction in vivo between tumour neoantigens and tumour antibodies could be a reason for the low frequency of antibody detection.

Materials and Methods

Tumour and corresponding normal tissues from the same individual were obtained from surgical specimens within half

an hour of excision. Venous blood samples were obtained from each patient providing the tissues as follows: sample 1, obtained preoperatively on the day of operation or on the previous day; sample 2, obtained three to six days postoperatively; sample 3, obtained 10 to 13 days postoperatively; and sample 4, obtained when the patient attended the follow-up clinic, two to four months postoperatively.

Four cellular fractions (nuclear, mitochondrial, microsomal, and supernatant) of both normal and carcinomatous tissue were tested by immunofluorescence, complement fixation, passive haemagglutination, and precipitation techniques against autologous sera in attempts to detect tumour-specific antibodies. The preparation of cellular fractions from tumour and normal tissue specimens, the preparation of serum, and the serological techniques used have been fully described elsewhere (Hodgkinson and Taylor, 1969; Taylor and Odili, 1970). Tissue specimens, sera, and tissue fractions were stored at -25°C until required. Tissues and sera from 36 patients were studied.

Results

Tumour-specific antibody responses were detected in 5 (14%) of the 36 cancer patients studied (Table I). In four of the five positive cases the autologous sera reacted against the nuclear

TABLE I—*Tumour-specific Antibody Detection in Sera of Cancer Patients and its Relation to Tumour Types*

Tumour Type	Number Studied	Antibody Detected	
		Number	% of Total No. of Cases
Carcinoma of breast ..	11	1	2.8
" oesophagus ..	2	0	0
" stomach ..	6	2	5.6
" colon ..	2	1	2.8
" rectum ..	8	1	2.8
" lung ..	4	0	0
" kidney ..	1	0	0
" ovary ..	1	0	0
" scrotum ..	1	0	0
Total ..	36	5	14

fraction of the tumours; in the fifth case the serum reactivity was directed against the microsomal fraction. Complement fixation was by far the best method of antibody detection. The positive sera in all five cases gave negative tests against the corresponding autologous normal tissue fractions—that is,

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