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HISTOCOMPATIBILITY TYPING FOR THE PREDICTION OF SUSCEPTIBILITY --ETC(U)
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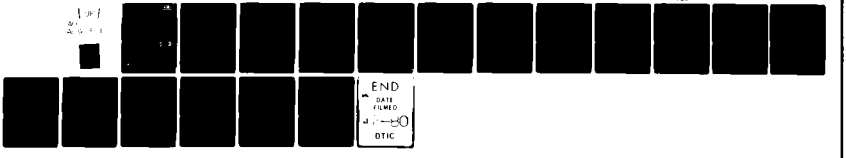

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# (6) Histocompatibility Typing for the Prediction of Susceptibility to Infectious Disease.

By

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A. ABSTRACT

The objective of ~~this contract is~~ to determine if susceptibility to certain infectious agents or morbidity associated with infection can be predicted based on knowledge of HLA type. ~~The contract was established to design and conduct~~ studies ~~that~~ will ~~indicate~~ if certain diseases or disease processes are associated with, or linked to, the HLA region genes. ~~The diseases, or complications~~ under study are ~~those selected by the project~~ officer for ~~investigation.~~ ~~In general~~ the format of these studies will follow ~~previously established~~ guidelines which include:

a. careful disease classification or subclassification based on firm clinical and laboratory parameters;

b. preparation of data collection forms based on items to be included in the analysis;

c. collection and storage of cells;

d. HLA typing of sample population (random populations exhibiting trait and/or families) and

e. statistical analysis for association or linkage of HLA and disease parameter.

During the first ten months of the project the existing HLA typing lab at Georgetown was expanded so as to have the capability of typing for HLA-A,B,C and DR locus alleles. This facility now has the capability of defining all World Health Organization Nomenclature Committee approved specificities in addition to a number of new antigens that have not yet been given a W.H.O. designation.

The population chosen for the index study comprises individuals who as infants suffered respiratory syncytial virus (RSV) infection, where a small number developed bronchiolitis requiring hospitalization while most recovered

with no severe side effects. Association and/or linkage between HLA, virus infection (seroconversion) and morbidity (bronchiolitis) as well as a number of other factors will be evaluated within the random sample population and within families using standard statistical methods.

A computer form has been custom designed to be used as a questionnaire to assure completeness of data collection in this study. The form was designed prior to initiation of the study to facilitate analysis following completion of data collection. This form was designed to serve as a model for future studies.

Families of patients with RSV disease who were willing to participate in the study have been identified through Children's Hospital, Washington, D.C., in cooperation with their private doctor. Blood samples have been obtained and the lymphocytes separated and cryopreserved on fifty-seven families (234 individuals). To date twenty-six individuals comprising six families have been HLA typed for A,B,C locus alleles. It is anticipated that all data will have been collected and preliminary analysis made during the fall of 1980 or early in 1981.

## B. INTRODUCTION

The following technical report summarizes the first ten months progress on ONR Contract N000-14-79-0886 "Histocompatibility Typing for the Prediction of Susceptibility to Infectious Disease". This project was initiated in August, 1979, to determine whether the histocompatibility type of an individual could predict his capacity to respond to a given infectious agent or protective vaccine in such a manner as to provide him with resistance for the particular disease process, and to determine if susceptibility to morbidity associated with certain infections is related to HLA type.

## C. BACKGROUND

The ability of individuals to operate in environments where they may be exposed to infectious agents may well depend on their ability not only to resist the disease process itself, but perhaps more importantly not to be susceptible to the more serious pathological consequences of an infection. The protective immune response to a variety of foreign agents (antigens) is handled by the immune system in an essentially similar manner whether the agent be virus, bacteria, parasitic, or transplanted tissue.

There is evidence to suggest that responses to infectious agents are under the control of genes in the major histocompatibility complex (MHC) of mammals. Some form of major histocompatibility complex appears to be present in all mammalian (and possibly chordate) species (1), thus, the MHC may play a vital functional role(s) which has allowed it to be conserved as a complex or "supergene" throughout evolution. The MHC in humans includes a series of structural and regulatory genes. Among these are the structural genes for HLA-A, B, C (class I) alloantigens (i.e. those made up of glycoprotein with attached $_2$ subunit), and at least two (2,3,4) and probably a minimum of three (5,6) B cell specific (class II) alloantigens (i.e. those made up of two glycoprotein chains) and, at least two types of T cell subpopulation alloantigens (7,8). In addition, there are lymphocyte activating determinants controlling stimulation in mixed lymphocyte culture (9), stimulation of primed lymphocytes (10), and stimulation of cytotoxic T cells (11). Genes controlling the production of various complement components (C2, C4, Bf), as well as genes that are the pivotal control points in both the classical and alternate pathways of complement activation (12, 13) are also found in the HLA region. Also, the response to various antigens in those mammals studied is under the control of inherited genes many of which reside

within the MHC (14). These genes have been termed "Immune response" or Ir genes. Although the presence of immune response genes is established in several mammalian species, there is only limited evidence for such genes in man. The evidence to suggest that humans have Ir genes comes from studies primarily in the area of responses to allergens, such as hayfever where it has been shown that the ability to become allergic is linked to genes within the human MHC (15).

We proposed to investigate in well controlled infectious disease models, whether in man the MHC genes contribute significantly to resistance or morbidity associated with infectious disease. These studies are intended to evaluate a number of disease systems.

Initially we chose to study a group of patients, all of whom suffered from respiratory syncitial virus (RSV) during the newborn period. This virus causes an upper respiratory infection and in some infants causes a serious complication with dyspnea and severe respiratory distress termed bronchiolitis which frequently is of such severity to merit hospitalization (16). The intent of the study is to determine whether there is a relationship (association or linkage) between HLA and those individuals who had severe consequences during the infection period. The study of this disease serves as a well controlled infectious disease model in which linkage between HLA susceptibility and disease may be evaluated and possibly even predicted. Such information could then be used to predict disease susceptibility in the young and perhaps give us some insight into the pathogenic mechanisms operating.

## D. RESEARCH DESIGN AND PLAN

The initial plans for this project were as follows:

1. Establishment of guidelines for use in designing and conducting HLA disease association studies, and development of a computer data information sheet in the form of a questionnaire, to be used as a model for these studies. Such a questionnaire would include (a) all personal information such as age, sex and family relationships, (b) pertinent clinical evaluations and laboratory studies (virus culture, antibody titer, etc.) necessary for adequate diagnosis and classification of the disease and (c) HLA typing data.

2. Establishment of a contract facility to perform histocompatibility typing for HLA-A, B, C, DR and other B cell antigens (for example, the recently described MB and MT antigens) at a reference research laboratory level.

3. Identification of patients and families willing to participate in the study. In the first of these projects, the patients would be those who suffered from the severe complication of respiratory syncitial virus (RSV) requiring hospitalization during the newborn period and their families (both parents plus at least one unaffected sibling).

4. Collection and cryopreservation of lymphocytes from the above patients and family members.

5. HLA-A, B, C, DR and D typing (when appropriate) of the cryopreserved lymphocytes from the above patients and family members.

6. Analysis of the association and/or linkage if any between the HLA complex and disease susceptibility to RSV disease and its complications.

## E. RESULTS

### 1. Development of Guidelines and Questionnaire-Computer Forms

Monthly meetings were held from August until December among investigators and key personnel at Georgetown, N.I.H. and Children's Hospital. The meetings were held to discuss the experimental design of the disease studies on a practical and statistical level. Statistical consultants were included in the discussions. (David Alling, Elizabeth Phillips and Nancy Mendell). Several points were evident from personal experience or based on the literature. If one is to ascertain association and/or linkage, one needs to be in agreement on the definition of the disease. Thus, experts on the particular disease to be studied should be included in the initial discussions and criteria for diagnosis and inclusion in the study determined. All available or attainable clinical evaluations and laboratory tests should be discussed and all data essential for unequivocal diagnosis and possible distinct subclassification should be selected and incorporated into the questionnaire computer form.

For association studies, the appropriate control population should be typed at the same time as the disease group, if there is not already an appropriate HLA typed control group. The control group should be drawn from the same geographic location and racial background. Age is not an issue unless the mean of the patient group is greater than 65.

For genetic linkage studies one needs more than 150 nuclear families, the most informative in this regard are those where both parents and several siblings can be studied. Since the most linkage information is obtained from families in which more than one individual has the disease, an effort should be made to identify such families and to obtain information on members in the extended family.

For the initial disease study, a number of parameters and their relevance to the study were considered. The results of several clinical assays which had already been performed were selected to be considered in the final analysis

(Virus isolated, serologic response to RSV, serologic response to other virus, RSV antibody). A custom made questionnaire-computer form was developed (see Appendix). Several drafts were necessary before the form was considered complete enough and clear enough to assure correctness and completeness of data entry. The form assures for collection of all previous test results which are to be considered in the final analysis. The format of the questionnaire was pre-designed to allow simplified computer data entry and expedite the final analysis. It is also intended to be used as a model for future studies. Guidelines were established for blood collection in children that were subsequently approved by the Human Use Committees of NIH, Georgetown University and Childrens Hospital.

2. Development of an HLA Histocompatibility Testing Serology Laboratory.

2.1 Establishment of HLA-A, B, C, DR Typing Trays.

Typing trays have been established within the laboratory at Georgetown which are capable of identifying all W.H.O. approved HLA-A,B,C and DR specificities and all specificities proposed in the 8th International Workshop pre-analysis (January 1980). In addition, B cell specificities not internationally recognized can be identified. Many of these are local designations. The specificities that have not received nomenclature committee approval are well defined but for various reasons (extremely low frequency, only one operationally monospecific antiserum, etc.) cannot be given designations. In total we can identify 18 alleles at the A locus, forty-one alleles at the B locus, nine at the C locus and 22 B cell antigens which probably define alleles at three loci (DR, MB, MT).

Establishment of reference quality typing trays requires several steps. (1) Selection of the best sera from colleagues and from workshops (International and American) based on correlation and specificity analysis of our own and pooled workshop data.

(2) Preparation of requests to colleagues for aliquots of the various sera. (3) Quality control the antisera in our own laboratory after it is received. Quality controlling entails testing the sera in serial dilutions (1:1-1:4) against 40 to 60 reference panel cells. This procedure provides information on the strength (titer) and thus to some extent the reliability of the reagent and tests for possible inactivation caused by shipment. (4) Tray Design. The selection of sera was performed and requests to colleagues were sent out in January. The quality control testing for the HLA-A, B, C sera was done in March and the tray design finalized and trays put out in April of 1980.

Establishment of B cell typing trays has an additional step. Many of the antisera used for B cell typing also contain anti-HLA-A, B or C antibodies which must be removed by absorption procedures. Platelets are most commonly used for the absorptions since they have HLA-A, B and C locus antigens on their cell surface and lack B cell antigens. Antisera requiring absorption to remove contaminating A, B, C antibodies are specifically absorbed when the specificity of the contaminating antibody is known. This requires making a large number of platelet preparations from specific donors. Antisera containing multiple HLA-A, B, C antibodies are absorbed with pooled packed platelets (pool = approximately 100 donors). Following absorption, all antisera are tested for residual HLA-A, B, C activity by the sensitive antiglobulin technique (17,18). Where feasible, since B cells have 5 to 6 times the density of HLA-A and B antigens than T cells, B cells negative for the B cell specificity and positive for the contaminating A, B, C specificity are used to test for completeness of absorption. It has been the experience of the principal investigator that specific absorption merits the effort involved. The absorption technique employed virtually eliminates non-specific absorption and assures removal of A, B, C antibodies below detectable levels. Often low frequency antibodies are

not removed with pooled packed platelets not to mention the amount of non-specific absorption and lack of standardization from one absorption to another. Each antiserum is then titered following absorption and used at an optimal dilution. At present, all B cell antisera have been absorbed and are ready to quality control for completeness of absorption of contaminating A,B,C antibodies and for titer. At least 1ml of each antiserum has been absorbed which will allow for a minimum of 900 typings.

## 2.2 Establishment of the Two-color Fluorescence Method for B Cell Typing.

The two-color fluorescence technique (19) was chosen to use for B cell typing because it requires a smaller amount of blood, a consideration of great importance when typing children. The technique does not require the separation of the B and T lymphocyte subpopulations. Basically, the procedure involves staining B cells with a fluorescein labelled anti-human immunoglobulin reagent. These stained cells are then used in a cytotoxicity assay where lysis is determined by the addition of ethidium bromide which stains the nuclei of dead cells red. The optics of the fluorescent microscope are adjusted so that one can see both red and green fluorescence simultaneously. Two scores are recorded when reading these plates, the % dead T cells and the % dead B cells. B cells are distinguished from T cells by their green fluorescein stained membrane. The T cell membrane appears as a dark ring.

The fluorescent microscope has been fitted with the appropriate filters to allow optimal detection of red and green fluorescence simultaneously. Standardization of the two color fluorescence technique against the technique that has been used by the principal investigator in the past will be performed simultaneously with quality controlling of the absorbed B cell antisera.

3. Identification and Selection of Patients and Family Members.

Over 10,000 patients with RSV disease have been studied in the Washington area over the past twenty-two years. Letters which explained the project were sent to the office of the attending physicians who were in turn asked to contact the parents of the patients and ask them if they and their children would be willing to participate in the study. The selection is intended to be as random as possible. The families who agreed were scheduled to come into Children's Hospital for blood sample collection. At this time they are personally seen by Dr. Kim, Senior investigator on the RSV study at Childrens Hospital. The computer-form-questionnaire is filled out by an attending nurse who has been assigned to the study.

If the family agrees, blood is obtained and immediately transported to a laboratory in the NIAID Division of the National Institutes of Health or Georgetown University for separation and cryopreservation of lymphocytes.

4. Cryopreservation of Lymphocytes from Patients and their Families

Essentially standard freezing and thawing techniques are used. These procedures have been in use at the Georgetown laboratory for the past four years. Since some of the lymphocytes were initially being frozen at NIH, a quality control experiment was designed to test the quality of the lymphocytes of several normal volunteers (including individuals who were known good responders) following freezing and thawing. Since the ability of lymphocytes to function in biological assays is the most critical test of their integrity, the following experiment was performed. Peripheral whole blood was drawn from six normal volunteers and the lymphocytes were separated and frozen at $5x10^6$ and $1x10^6$/ml. Frozen vials from each donor were thawed and the yield and % viability was ascertained by trypan blue exclusion. In addition, the capability of the frozen/thawed lymphocytes to respond to PHA and to a

lymphocyte stimulator pool was determined. Results of the tests performed on the vials frozen at $5x10^6$ are given in the table below. Vials frozen at $1x10^6$ were thawed at the same time and check for viability. All the cells were in excellent condition.

| CELL | YIELD/VIAL ($X10^6$) | % VIABILITY | CONTROL 1* | PHA | CONTROL 2 | STIMULATOR POOL 1 |
|---|---|---|---|---|---|---|
| H1 | 2.28 | 98 | 194 | 37,002 | 272 | 9,100 |
| H2 | 2.23 | 100 | 108 | 38,997 | 153 | 11,510 |
| H3 | 2.01 | 96 | 116 | 22,892 | 207 | 18,157 |
| H4 | 2.26 | 90 | 210 | 33,502 | 628 | 13,797 |
| H5 | 2.25 | 94 | 392 | 32,927 | 1839 | 17,268 |
| H6 | 2.52 | 96 | 280 | 31,704 | 794 | 19,563 |

* These values are all median cpm of triplicate values.

The quality control of the freezing procedure was performed before any lymphocytes from patients and families were frozen for the study.

To date, blood samples have been obtained and the lymphocytes separated and cryopreserved on fifty seven families, a total of 234 samples.

5. HLA Genotyping

5.1 HLA-A,B,C Typing. HLA genotyping typing for antigens of the A,B, and C allelic series has been accomplished in 26 individuals constituting six families. HLA phenotypes are given in Table 1. Technically excellent results were obtained. No analysis or statements can be made about the results obtained on such a small number of individuals.

TABLE 1

| Family # | Member | A locus | B locus | C locus | Genotypes |
|---|---|---|---|---|---|
| 8 | Mother | A3, A24 | B39, B44 (w4,w6) | Cw5 | A3, B39, w6, Cblank/A24, B44, w4, Cw5 |
| | Father | A28, A23 | B45, B17.2 (w4,w6) | Cw6 | A28, B45, w6, Cw6/A23, B17.2, w4, Cblank |
| | Sib A | A3, A28 | B39, B45 (w6) | Cw6 | A3, B39, w6, Cblank/A28, B45, w6, Cw6 |
| | Sib B | A23, A24 | B44, B45 (w4,w6) | Cw5 | A24, B44, w4, Cw5/A23, B45, w6, Cblank |
| 9 | Mother | A1, A3 | B45, B53 (w4,w6) | Cw4 | A1, B53, w4, Cw4/A3, B45, w6, Cblank |
| | Father | A23, A2 | 4C, B45 (w6) | Cw2 | A23, 4C, w6, Cw2/A2, B45, w6, Cblank |
| | Sib A | A3, A23 | B45, 4C (w6) | Cw2 | A3, B45, w6, Cblank/A23, 4C, w6, Cw2 |
| | Sib B | A3, A23 | B45, 4C (w6) | Cw2 | A3, B45, w6, Cblank/A23, 4C, w6, Cw2 |
| 11 | Mother | A2, A3 | B8, B14 (w6) | T8 | A2, B8, w6/A3, B14, w6, T8 |
| | Father | A2, A25 | B18, B37 (w4/w6) | Cw6 | A2, B37, w4Cw6/A25, B18, w6 |
| | Sib A | A2, A25 | B8, B18 (w6) | - | A2, B8, w6/A25, B18, w6 |
| | Sib B | A2, A25 | B8, B18 (w6) | - | A2, B8, w6/A25, B18, w6 |
| 13 | Mother | A1, A24 | B8, B44 (w4/w6) | Cw5 | A1, B8, w6, Cblank/A24, B44, w4, Cw5 |
| | Father | A32, A2 | B22, B51 (w4/w6) | Cw3 | A32, B22, w6, Cw3/A2, B51, w4, Cblank |
| | Sib A | A24, A32 | B44, B22, (w4,w6) | Cw3,Cw5 | A24, B44, w4, Cw5/A32, B22, w6, Cw3 |
| | Sib B | A2, A24 | B51, B44 (w4) | Cw5 | A2, B51, w4, Cblank/A24, B44, w4, Cw5 |
| | Sib C | A1, A32 | B8, B22 (w6) | Cw3 | A1, B8, w6, Cblank/A32, B22, w6, Cw3 |
| 19 | Mother | A3, A26 | B7, 8w59(w6) | Cw3 | A3, B7, w6, (Cw3)/A26, 8w59, w6, Cw3 |
| | Father | A2, A33 | B40, Bw53(w4/w6) | Cw3,Cw4 | A2, B40, w6, Cw3/A33, Bw53, w4, Cw4 |
| | Sib A | A2, A3 | B7, B40(w6) | Cw3 | A3, B7, w6, Cw3/A2, B40, w6, Cw3 |
| | Sib B | A2, A3 | B7, B40(w6) | Cw3 | A3, B7, w6, (Cw3)/A2, B40, w6, Cw3 |
| | Sib C | A26, A33 | 8w59, Bw53 (w4,w6) | Cw3,Cw4 | A26, 8w59, w6, Cw3/A33, Bw53, w4, Cw4 |
| 55 | Mother | A2, A2 | B18, B27(w4/w6) | Cw1 | A2, B18, w6/A2, B27, w4 Cw1 |
| | Father | A1, A28 | B7, B8 (w6) | blank | A28, B7, w6/A1, B8, w6 |
| | Sib A | A2, A28 | B7, B27 (w4/w6) | Cw1 | A28, B7, w6/A2, B27, w4, Cw1 |
| | Sib B | A2, A28 | B7, B18 (w6) | blank | A28, B7, w6/A2, B18, w6 |

5.2 HLA-DR typing. HLA-DR (B cell) typing will begin in June after completion of the quality control of the antisera and the two-color fluorescence technique.

F. PROPOSAL FOR CONTINUATION OF CONTRACT. WORK PLAN.

1. Histocompatibility Typing for RS Virus Association

During the next contract year we will continue to accumulate patients in the RS study and to type them for HLA-A, B, C and DR specificities. It is anticipated that the statistical analysis can begin early in 1981 to evaluate associations and the possibility of linkage between HLA type and severe clinical illness. At this time any informative families will be identified for HLA-D typing. Concomitantly, a normal control panel of American Blacks from the Washington, D.C. area will be HLA typed to serve as control group for the present and future studies involving Blacks.

2. It is anticipated that during the next contract year two studies will begin, following the same format as the index study.

2.1 Leishmania Study

HLA typing will be performed on frozen cells from individuals with a unique form of cutaneous Leishmania. The study will include a pilot group to test for cell viability and functional capacity following shipment of cells to our laboratory, the typing of index cases, families, and control populations of sufficient number for appropriate statistical analysis.

2.2 Hepatitis B Study

HLA typing will be performed on frozen cells from individuals receiving Hepatitis B vaccine, where there are known high and low responders, and the antigen has been well characterized. The donors will be from the random population and an association between HLA and seropositive response to hepatitis vaccine will be evaluated.

G. PUBLICATIONS AND POSTERS

During the first ten months of this contract one manuscript has been accepted for publication and five abstracts have been accepted for poster presentations.

Publication

1. Mendell, N.R., Johnson, A.H., Ward, F.E., Hartzman, R.J., Phillips, E.A., Ayres, J., Amos, D.B. and Ciftan, E.A. Statistical methods for studying homozygous typing cells of unknown specificity. 1980. Human Immunology (in press).

Posters:

1. Ward, F.E., Hartzman, R., Willoughby, P.B., Amos, D.B. and Johnson, A.H. Evidence for multiple genes and multiple alleles in the D-DR region: The D-DRw4 complex defined by HTC typing and B cell serology. 8th International Histocompatibility Workshop and Conference, 1980.

2. Johnson, A.H., Hartzman, R.J., Grier, J.O., Amos, D.B., Mann, D.L. and Ward, F.E. DRw6 - Two serologically distinct specificities associated with Dw6 and Dw9. 8th International Histocompatibility Workshop and Conference, 1980.

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4. Hartzman, R., Pappas, F., Johnson, A., Ward, F. and Amos, D.B. B-cell serology, HTC and PLT: Analysis of HLA-DW and DRw5,6 and DuB31. 8th International Histocompatibility Workshop and Conference, 1980.

5. Eckels, D.D., Hartzman, R.J., Lamb, M., Ward, F.E., Johnson, A.H. and Amos, D.B. Possible new HLA-D specificities. 8th International Histocompatibility Workshop and Conference, 1980.

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