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Failure of Lymphocyte-Membrane HLA-A and -B Expression in Two Siblings with Combined Immunodeficiency

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A diagnosis of partial combined immunodeficiency was made in two Turkish siblings with a history of multiple pyogenic infections and persistent candidiasis. They demonstrated severe hypo-γ-globulinemia, with B-lymphocytes, but deficient plasma cell differentiation. T-Lymphocytes were decreased in number and did not respond to antigens, but did proliferate in cultures with lectins and allogeneic cells. HLA-A and -B determinants were not detected on blood lymphocytes, but they were expressed by cultured lymphoblasts, cultured fibroblasts, and were present in serum. MLR-Stimulatory capacity was intermediate and only two of six anti-HLA-DRw7 antisera demonstrated B-cell reactivity. β-2-Microglobulin (B2M) was not detected on the surface of T-lymphocytes, but was found in cross-sectioned T-cell membranes. B-lymphocytes carried B2M normally. The absence of HLA-A and -B determinants on lymphocytes of patients with similar immunodeficiency syndromes suggests a role for HLA determinants in lymphocyte differentiation.

INTRODUCTION

Primary combined immunodeficiencies in man are a heterogeneous group of disorders in which the immune response can be blocked at various stages of differentiation of T- and B-lymphocytes (1, 2). Recent investigations support the view that defective T-cell maturation leads to deficient B-cell response to antigenic stimulation (3). Animal experiments have revealed the important role of gene products of the major histocompatibility complex (MHC) in interactions between T-cells, B-cells, and macrophages (4). A role of HLA determinants in human T-cell differentiation in the thymus has been suggested recently (5). Therefore, abnormal expression of MHC products could be expected to be related to the development of immunodeficiencies. The clinical and laboratory data of two siblings with partial combined immunodeficiency and no detectable HLA antigens on their lymphocytes are reported.[2] A similar patient has been described recently (8).

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[2] Short descriptions of these patients have been presented previously by the authors (6, 7).

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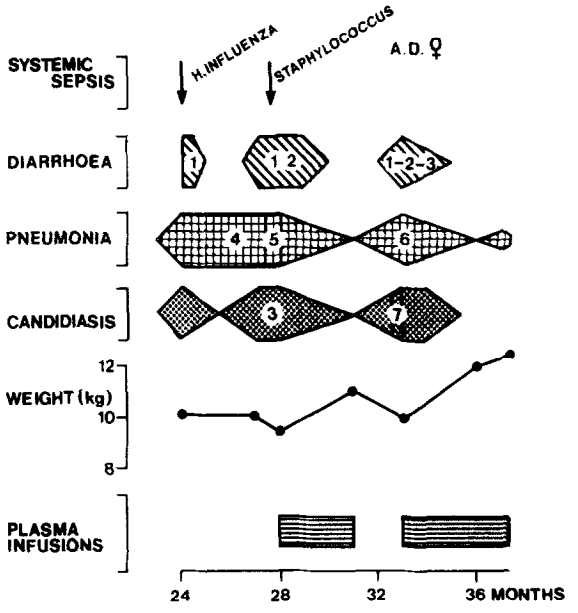


FIG. 1. Schematic representation of the clinical course of patient A.D. The arrows indicate episodes of systemic sepsis. The numbers represent the microorganisms cultured during each episode of infection: (1) *E. coli:* $>10^6$ microorganisms/ml duodenal fluid, (2) *Gardia lamblia*, (3) *Candida albicans*, (4) *S. aureus*, later on: *Klebsiella*, (5) *Pneumocystis carinii* (probably), (6) *H. influenza*, *Pseudomonas aeruginosa*, (7) Herpes simplex hominis, type II.

MATERIALS AND METHODS

Family History

The family of the two children reported here originated from an isolated village in Turkey. There were four children: the eldest, a female, died in Turkey at 4 months; the second child, a male, remains in good health; the two other children (patient A.D. and patient K.D.) will be described.

Patient A.D. This female child was admitted at 12 months of age to the Sophia Children's Hospital, Rotterdam. Gram negative septicemia (*Enterococcus*), intractable diarrhea (*Escherichia coli, Candida albicans, Giardia lamblia*), and extensive mucocutaneous candidiasis were diagnosed. At age 2 years she had several bouts of bronchopneumonia (*Staphylococcus aureus, Klebsiella, Haemophilus influenza*) and interstitial pneumonitis (Fig. 1). She had a further two episodes of septicemia (*H. influenza, S. aureus*). The symptoms were relieved only when antibiotic treatment was supported by plasma therapy. Necrotizing lesions on the nates associated with herpes simplex hominis type II occurred once. There was no evidence of systemic spread of viruses. Patient A.D. died at age 3 from a severe attack of bronchiolitis and bronchopneumonia. At necropsy normal-sized tonsils and lymph nodes were found but the thymus weighed 5 g (normal 20–30 g).

Patient K.D. The youngest child, a boy, was admitted at 5 months of age, because of relapsing pneumonia (*S. aureus, H. influenza, Klebsiella*). He also

developed recurrent diarrhea (*C. albicans*, *G. lamblia*), mucocutaneous candidiasis, and systemic sepsis (*Klebsiella*). These symptoms subsided on antibiotic therapy after ordinary plasma therapy was started. However a pneumonitis persisted. Open-lung biopsy revealed no *Pneumocystis carinii*. Patient K.D. died at age 3 due to rapidly developing bronchiolitis.

Both patients had severe growth retardation after the age of 4 months, but growth rates improved after plasma therapy was initiated. Both had slightly blue sclerae, thick black hair, and normal nails. Lymph nodes were palpable, and tonsils were present. Radiological examination showed normal thymic shadows, a normal-sized spleen, and no skeletal abnormalities.

Serological Studies

These investigations were performed on sera obtained before plasma therapy.

Immunoglobulins. Quantitation of immunoglobulins G, G 1–4, M, A, and D in serum and of IgA and secretory piece in saliva was performed by single radial immunodiffusion. Normal ranges were derived from age-related reference groups, investigated by comparable (9) or the same (10) antisera. IgA in serum was also measured by radioimmunoassay (11). IgE levels in serum were compared with a reference serum in a radioimmunoassay (12).

Complement. Various complement components were investigated in either quantitative single radial immunodiffusion (C1q, C4, C3, C5, and Factor B) or functional assays (Factor B, C2).

Lymphocytotoxic antibodies. The presence of serum antibodies against lymphocytes was assayed by indirect immunofluorescence (13), agglutination (14), and microcytotoxicity methods (15), using a panel of donor lymphocytes.

Granulocyte Functions

The function of granulocytes was measured by the uptake of immune complexes (16), oxidative metabolism (16), and intracellular killing of bacteria (17).

Immunofluorescence Studies

Cytoplasmic fluorescence on bone marrow cells and membrane fluorescence on peripheral blood lymphocytes (PBL) were performed using methods and antisera described by Vossen (18). Normal ranges were derived from age-related reference groups (18). In order to detect all sIg-positive B-lymphocytes in PBL a goat-anti-Fab-antiserum with specificity for Fab fragments of all Ig classes was labeled with fluorescein (FITC) (19).

HTLA-Human thymus-lymphocyte antigen. In order to detect human thymus-lymphocyte antigen (HTLA) on PBL and on tissue cells, respectively, the antisera described by Asma *et al.* (19) and by Brutel de la Riviere *et al.* (20) were used. The first antiserum was directly labeled with rhodamine (TRITC). When used in a double-staining method, the cells were first incubated with anti-Fab-FITC and then incubated with anti-HTLA-TRITC. In normal PBL 1% of the cells show double staining, and 2% do not stain with either antiserum. Control values were derived from age-related reference groups (19). The second HTLA-antiserum was applied to tissues in an indirect fluorescence assay. The first step, a 1:20 dilution

of the HTLA-antiserum was followed by a 1:75 dilution of swine-anti-rabbit IgG-FITC (21). Tissue fluorescence was carried out by methods previously described (21).

β-2-Microglobulin (B2M). Two anti-β-2-microglobulin antisera were used. One, kindly provided by Dr. Radl, REPGO-TNO, Ryswyk, The Netherlands, was prepared by immunizing a rabbit with B2M isolated from human urine. This antiserum had the following specificities in membrane fluorescence assays: normal PBL—100% of the cells positive; acute lymphoblastic leukemia cells—100% positive; erythroid cells—negative; epithelial cells of a bladder tumor—negative; mouse spleen lymphocytes—negative. This antiserum was labeled with TRITC and used in 1:100 dilution in the membrane fluorescence as well as tissue fluorescence studies.

When used in a double-staining technique, the cells were first incubated with anti-B2M-TRITC and then with anti-Fab-FITC. The number of double-marked cells was counted and expressed as a percentage of the B2M-positive cells. When the degree of staining by the fluorescent anti-B2M serum was compared, the same numbers of patient's cells and normal PBL, incubated with the same amount of antiserum, were used in parallel. This anti-B2M serum was also radiolabeled with ^{125}I to measure the difference in B2M expression on patient's lymphocytes and control PBL. The second anti-B2M serum, obtained from Dakopatts, Denmark (Batch 10-472), was applied in an indirect fluorescence technique on tissues in a dilution of 1:100, followed by a 1:120 dilution of swine anti-rabbit IgG-FITC.

Cross-section of blood lymphocytes. In order to be able to visualize the inner side of membranes of peripheral blood T-lymphocytes, PBL were immersed in gelatin, snap-frozen in liquid nitrogen, and cut into 4-μm cryostat sections (22). This cross-section of PBL was compared with PBL in suspension, using both anti-B2M antisera in parallel.

Cell Membrane Markers Detected by Rosette Techniques

Other cell membrane markers were investigated using sheep red blood cells (E rosettes), human D^+ red blood cells sensitized with anti-D serum (EA rosettes) (23), and sensitized sheep red blood cells coated with mouse complement (EAC rosettes) (24).

Cell-Mediated Immunity

Skin tests. In vivo cell-mediated immunity was investigated by a dinitrochlorobenzene (DNCB) patch test using various amounts of DNCB (1-, 3-, 10-, and 30-μg DNCB) 3 weeks after sensitization, and by intracutaneous tests with PPD, *Candida* antigen, mumps antigen, *Trichophyton*, and streptokinase–streptodornase (SK-SD).

Lymphocyte transformation tests. In vitro proliferation of lymphocytes in response to stimulation with lectins, anti-lymphocyte serum (ALS), allogeneic cells (MLR), and Candida antigen was assayed as described by Schweitzer *et al.* (25) and DuBois *et al.* (26). Cultures in triplicate were performed in microtiter plates. The lectins used were: phytohemagglutinin (PHA), Pokeweed mitogen (PWM), and concanavalin A (Con A). Cells were pulsed 16 hr before harvesting with 0.4 μCi [^{3}H]thymidine (Radiochemical Center, Amersham). Results were expressed

in counts per minute (cpm) thymidine incorporation per 4×10^4 lymphocytes. In the MLR, stimulator cells were either treated with mitomycin or irradiated (2500 rad). Control values were derived from the response of PBL of two healthy donors cultured in parallel. The *in vitro* response of patient K.D.'s PBL to stimulation with diphtheria toxin and tetanus toxin was investigated 3 weeks after a booster injection with these antigens.

Antibody-dependent cytotoxicity (ADC). The killing capacity of patients' PBL was assayed using sensitized target cells derived from a melanoma cell line (24).

Cell-mediated lympholysis (CML). The *in vitro* development of cytolytic capacity was investigated in a cell-mediated lympholysis assay (CML) (27). PBL of donor X were first stimulated by irradiated PBL of donor Y in a mixed lymphocyte culture. In parallel, PBL of donor Y were transformed to lymphoblasts by culture in the presence of PHA. The cytotoxic capacity of stimulated X cells was then assayed using ^{51}Cr-labeled Y-lymphoblasts as targets (27). This CML assay was used in two ways: first, to measure the cytotoxic capacity of stimulated K.D. lymphocytes against nonsensitized unrelated donor lymphoblasts, and second, to investigate whether unrelated donor lymphocytes were able to lyse patient K.D.'s lymphoblasts. Thus, because HLA-A and -B determinants on the target cells have been shown to play a role in the lytic phase of the CML (27), this second mode of the CML was used to detect HLA antigens on patients' lymphoblasts. The lysis of target cells was measured and calculated according to the formula:

$$\frac{\text{cpm release in experiment} - \text{cpm spontaneous } ^{51}\text{Cr release}}{\text{cpm maximum release} - \text{cpm spontaneous release}} \times 100.$$

The cpm represented the release of ^{51}Cr from 10^4 target cells (27).

PWM induced B-cell maturation. In vitro differentiation capacity of patients' PBL was investigated in a PWM induced T-cell dependent B-cell maturation assay (28). After 10 days culture with PWM the cultured cells were spun in a cytocentrifuge and fixed and stained with fluorescinated anti-immunoglobulin sera. The numbers of positive staining cells were enumerated by counting 10^3 blasts cells. Control values were derived from experiments on control PBL, run in parallel.

Immunogenetic Studies

Techniques used to detect HLA determinants. HLA-A, -B, and -C determinants were investigated by the standard typing technique with the NIH lymphocytotoxicity method using 120 different HLA antisera (15), and by an agglutination assay in EDTA blood performed with 30 different HLA antisera (14). The standard cytotoxicity method was also applied to E-rosette depleted B-cell enriched PBL and to PBL which had first been incubated for 24 hr in tissue culture medium supplemented with 20% pooled human serum. The HLA phenotypes of the parents and the plasma donors were determined by the standard HLA typing technique. Because every patient had one plasma donor only, the following assays were performed using antisera against HLA determinants, predicted from the phenotypes of the parents or donors.

HLA determinants on platelets. HLA determinants on platelets were investi-

gated by a platelet complement fixation test (29) and a platelet inhibition assay. In the last assay platelets of both patients were used to adsorb HLA antisera, after which the adsorbed antisera were investigated in the standard lymphocytotoxicity test against a pool of donor lymphocytes (30).

HLA determinants on lymphoblasts. Patients' lymphocytes were transformed to lymphoblasts by 3-day culture in the presence of PHA, after which the lymphoblasts were labeled with ^{51}Cr. These target cells were incubated with HLA antisera and then with normal PBL effector cells, after which the ^{51}Cr release from the target cells was measured (31).

HLA antigens in serum. The presence of HLA antigens in serum was investigated by using radiolabeled HLA antisera in an adsorption–inhibition assay with a panel of typed donor lymphocytes (32).

HLA-determinants on fibroblasts. Fibroblasts of both patients were cultured for 6 weeks and assayed by a selected panel of HLA antisera, used in an indirect immunofluorescence assay (13), a cytotoxicity method using fluorochromatic labeled fibroblasts (33), and a ^{51}Cr release assay (34).

B-Cell allotypic determinants. Enriched B-cell suspensions were tested with 45 antisera against B-cell allotypic determinants (DR-determinants) in an indirect fluorescence method (13).

Additional Assays

Thymus-dependent serum factor (SF) was assayed by methods described by Astaldi *et al.* (35). Karyograms were made according to routine techniques, including the Reverse, Atebrine, and Giemsa banding assays.

RESULTS

Immunological Investigations

Immunoglobulins, Antibodies, and Complement Components

Severe hypo-γ-globulinemia was diagnosed in both patients (Table 1). All immunoglobulin classes and subclasses were below the normal level for age except for a normal level of IgM in the serum of patient A.D. In saliva no IgA could be found whereas a normal amount of secretory piece was present. No titers of isohemagglutinins, nor antibodies against a variety of bacteria, parasites, fungi, viruses, or various tissues were found. No antibodies against allogeneic lymphocytes were detected. Complement components (C2, C4, C3, Factor B, C5) showed normal levels with exception of slightly lowered levels of C1q (60% of normal).

Cellular Immunity

Hematology. In each patient leukocyte counts were about half the normal age-related value. Absolute lymphocyte counts varied between 1.0 and 3.0×10^9 per liter (age-matched controls: $5.0-7.0 \times 10^9$ per liter). Neutrophils were abnormal in size and morphology, suggesting disturbed myeloid cell maturation (36). Functional assays of granulocytes revealed normal values. Absolute monocyte counts were slightly decreased, whereas numbers of eosinophils were consistently increased.

Plasma cells and B-lymphocytes. Bone marrow biopsies showed only few plasma cells. These were shown to contain IgM mainly (Table 2). Only patient

TABLE 1
IMMUNOGLOBULINS (mg/100 ml)

Ig-(Sub)class[a]	Patient A.D. ♀	Normal range	Patient K.D. ♂	Normal range
G	<60	350–1140	<40	140–930
G1	50	350–1180	—[b]	100–850
G2	<15	75–210	—	60–200
G3	12	15–145	6	5–160
G4	<2.5	<2.5–90	—	<2.5–90
A	0.11	13–100	0.13	4–80
Sc-A	<0.5	>30	—	>20
Sc-piece	6.4	4–20	—	2–25
M	80	40–230	4	20–120
D	<0.7	0–3	<0.7	0–2.5
E (IU/ml)	<1	10–500	<1	10–500

[a] Sc-A and Sc-piece, secretory IgA and secretory piece.
[b] Not determined.

A.D. had normal numbers of IgM-containing cells. The IgM cells were plasma cells, morphologically and by fluorescent staining. In PBL suspensions of both children, B-lymphocytes, bearing Ig of various classes were detected (Table 2). With an anti-Fab-antiserum 40% of PBL were shown to be B-lymphocytes (see also Table 9). The relative high percentages of B-lymphocytes were shown to reflect normal absolute numbers when corrected for the lymphopenia. Slightly elevated percentages of Fc-bearing lymphocytes were detected by EA-rosette assay: 31 ± 4% (controls 26 ± 3%), whereas complement receptor-bearing lym-

TABLE 2
CYTOPLASMIC IMMUNOGLOBULINS IN BONE MARROW CELLS

IgCC[a]	Patient A.D. ♀	Normal range	Patient K.D. ♂	Normal range
G	10	35–268	0	33–151
A	0	49–348	0	10–163
M	226	34–203	7	16–272
D	3	0–20	0	0–27

SURFACE IMMUNOGLOBULINS ON BLOOD LYMPHOCYTES

IgBC[b]	Patient A.D. ♀	Normal range	Patient K.D. ♂	Normal range
G	6	1–10	7	0–25
A	0	0–2	1	0–4
M	22	7–22	18	13–31
D	33	8–31	22	15–41

[a] IgCC, immunoglobulin-containing lymphocytes and plasma cells; numbers are expressed per 10^5 lymphocytes and plasma cells.
[b] IgBC, immunoglobulin-bearing cells; expressed in numbers of Ig-positive cells per 100 lymphocytes.

phocytes were slightly decreased: 16 ± 10% (controls 23 ± 4%). T-Lymphocyte percentages were 50–60% (control 70–90%) when investigated by E-rosette and anti-HTLA fluorescence assays (see also Table 9). This implied that absolute T-lymphocyte numbers were about one-third of the normal values for age.

Histopathology of the Immune System

Thymus. Thymic biopsy of patient K.D. showed decreased numbers of Hassall's corpuscles and of cortical lymphocytes. The architecture of cortex and medulla was essentially normal. By fluorescence the thymic cortex contained few HTLA-positive cells (Table 3). Postmortem histology of A.D.'s thymus showed atrophy with Hassall's corpuscles. The cortex was depleted of lymphocytes with few HTLA-positive cells. Thus, both children had a lymphocyte-depleted but essentially normal thymus. The atrophy of A.D.'s thymus appeared to be caused by agonal stress.

Lymph nodes. Lymph nodes of both patients, excised shortly after severe infection, had normal weight and architecture. There were few follicles and no germinal centers. Sporadic plasma cells, scattered through the medulla and through rare follicles, contained only IgM and IgD. The paracortical areas were depleted of lymphocytes, but showed some clusters of HTLA-positive cells.

Spleen. Splenic tissue, obtained at necropsy of patient A.D., showed underdeveloped periarteriolar lymphocyte sheaths containing HTLA-positive cells. Clusters of lymphocytes, staining positively for κ- and λ-chains, appeared to be organized follicles. These scarce follicles, without germinal-center reaction, contained some plasma cells, positive for IgM and IgD only.

Intestinal wall. There were few Peyers' patches in both patients. The intestinal wall contained scarce follicles with no center reactions. Plasma cells, containing IgM and IgD only, were occasionally found in these follicles and throughout the intestinal wall.

TABLE 3
IMMUNOHISTOPATHOLOGY

	Immunoglobulin-containing lymphocytes and plasma cells[a]					
	Thymus	Spleen A.D. ♀	Lymph nodes A.D. ♀	Lymph nodes K.D. ♂	Intestinal wall	Lung A.D. ♀
IgG	–	(+)	–	–	–	(+)
IgA	–	–	–	–	–	–
IgM	–	+	+	(+)	+	+
IgD	–	+	+		+	+
κ and λ	–	+	+	(+)	+	+
	Membrane pattern of immunofluorescence					
HTLA	+	+	+	+	+	+
B2M	+	+	+	+	+	

[a] Numbers of immunoglobulin-containing cells and plasma cells were classified as follows: –, negative, no positive staining cells found; (+), dubious, only a few cells found; +, positive, but lower numbers of cells than normal; ++, positive, normal numbers of cells. Membrane pattern of immunofluorescence: lymphoid cells with a ring of fluorescence around the cells.

TABLE 4
LYMPHOCYTE TRANSFORMATION TESTS[a]

	Patient A.D. ♀	Patient K.D. ♂	Control
PHA	27.4 (2.7)	16.7 (1.8)	24.3 (3.6)
PWM	10.6 (1.1)	6.4 (1.1)	7.1 (1.1)
Con A	7.9 (1.5)	7.8 (1.6)	8.0 (1.2)
ALS	18.3 (1.2)	16.9 (2.0)	17.8 (1.9)
Ø[b]	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)
MLR-R[c]	11.2 (1.5)	9.9 (1.4)	12.4 (1.4)
Candida	0.4 (0.3)	0.2 (0.2)	3.5 (2.6)
Diphtheria		0.3	5.6
Tetanus		0.2	7.2

[a] Mean and (standard deviation) of at least four separate experiments.
[b] Ø, No mitogen added.
[c] MLR-R, MLR of patients' lymphocytes in response to allogeneic lymphocytes.

Lung. Although patient A.D. had severe recurrent lung infections, she had only a few plasma cells, most containing IgM and few IgG.

Cell-Mediated Immunity in Vivo

Skin tests with DNCB were dubious in both children when challenged with 30 μg DNCB only. Negative intracutaneous skin tests were obtained with a variety of antigens including *Candida* antigen.

Lymphocyte Functions in Vitro

Lymphocyte transformation tests. Lymphocyte proliferation was normal when stimulated by lectins, ALS, and allogeneic cells (Table 4). Although both patients had extensive mucocutaneous candidiasis, their lymphocytes did not proliferate in culture with *Candida* antigen. A booster injection of diphtheria and tetanus was not followed by an *in vitro* lymphocyte response to these antigens.

Cytolytic capacity in vitro. Patients' PBL showed normal cytolytic activity against sensitized target cells (ADC). In the CML assay K.D.'s PBL were capable of developing cytotoxicity against unrelated donor lymphoblasts (Table 5) ($P + X_R \rightarrow X$).

T-Cell dependent B-cell differentiation in vitro. PWM induced B-cell maturation was almost absent, although a few lymphoblasts were shown to contain IgM (Table 6). Low numbers of Ig-containing cells did not exceed 15% of the number of control PBL.

Immunogenetic Studies

HLA-A, -B, and -C Determinants

Lymphocytes. On lymphocytes of both patients no HLA-A, -B, and -C determinants could be detected with all HLA antisera available using the standard typing technique and an agglutination assay (Table 7). The standard typing technique was also applied to patients' PBL, after 24 hr culture in medium, to exclude covering of HLA determinants by (auto)antibodies, and to enriched B-lymphocyte suspensions, with negative results.

TABLE 5
CELL-MEDIATED LYMPHOLYSIS[a]

Effector[b]	Target	Ratio		
		1/12.5	1/6.25	1/3.12
$P + X_R$	P	1.1	1.3	
	X	11.1	2.6	
$X + R_R$	P	11.0	6.1	4.1
	X	3.1	1.9	0.6
$X + Y_R$	Y	17.4	12.1	
	X	4.0	2.3	

[a] HLA phenotypes = donor X: A3, W19, B7, W17; donor Y: A2, 11, BW40, 15.
[b] P, patient K.D., X and Y, unrelated donors. P_R, X_R, Y_R: Irradiated stimulator cells in the MLR phase of the CML assay.

Further assays were performed selectively for HLA determinants predicted from the phenotypes of the parents and plasma donors (Table 8).

Platelets. Platelets of both patients were shown to be negative for HLA determinants, in a platelet complement fixation test. However, by the more sensitive platelet adsorbtion inhibition assay some HLA antigens were shown to be present but the amount of HLA antigens detected in this way appeared low.

Cultured lymphoblasts. When patients' lymphocytes were first transformed to lymphoblasts and then investigated by HLA antisera in a lymphocyte-dependent cytotoxicity assay, the lymphoblasts were shown to express some HLA determinants. This assay was not reliable, because the possibility of false positive reactions due to extra antibodies in the reagents used could not be excluded. However, support for the expression of HLA determinants on transformed patients' lymphoblasts came from the CML studies: MLR-Stimulated unrelated donor lymphocytes were shown to develop cytotoxicity against lymphoblasts of patient K.D. (Table 5) ($X + P_R \rightarrow P$).

Serum. In contrast to the cell membrane studies, the patients' sera contained HLA antigens. Only HLA-A locus derived antigens were detected. Because the patients were on plasma therapy at that time, the HLA-A3 and A9 antigens in patient A.D.'s serum could theoretically have been derived from her plasma donor. However, donor antigens were not detected in K.D.'s serum (e.g., A2 in K.D.). Furthermore, when plasma transfusions were interrupted for 3 weeks the

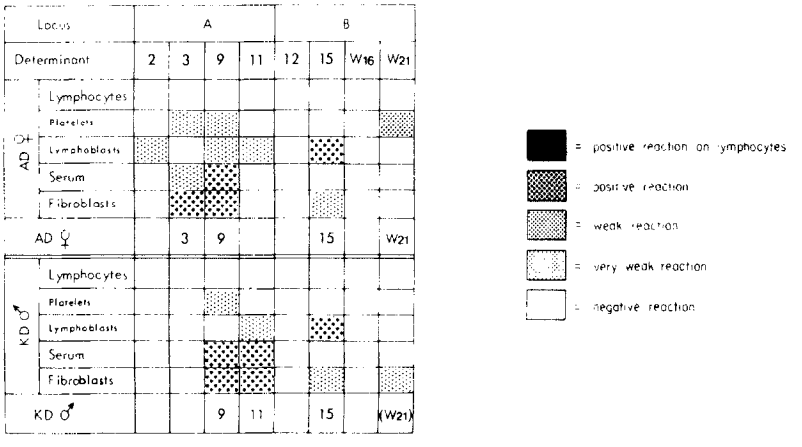
TABLE 6
PWM INDUCED B-CELL MATURATION[a]

	Patient A.D.	Patient K.D.	Control	
	+PWM	+PWM	+PWM	−PWM
Ig[b]	15	15	125	<5
IgM		5	25	
IgA	2	<5	45	

[a] Values are expressed in numbers of Ig-containing cells per 10^3 blast cells.
[b] Ig, immunoglobulins, all classes.

TABLE 7

ATTEMPTS TO DETECT HLA ANTIGENS[a]



[a] *Lymphocytes:* Standard HLA typing on lymphocytes, on enriched B-cell suspensions, and on PBL after 24 hr incubation in culture medium and typing by an EDTA agglutination assay. *Platelets:* Platelet complement fixation and platelet inhibition assays. *Lymphoblasts:* PHA-induced lymphoblasts investigated by an antibody-dependent cytotoxicity method. *Serum:* HLA antigens in serum, detected by radioimmunoassay. *Fibroblasts:* Indirect immunofluorescence, and fluorochromatic-labeled or ^{51}Cr-labeled target cell release in a cytotoxicity assay.

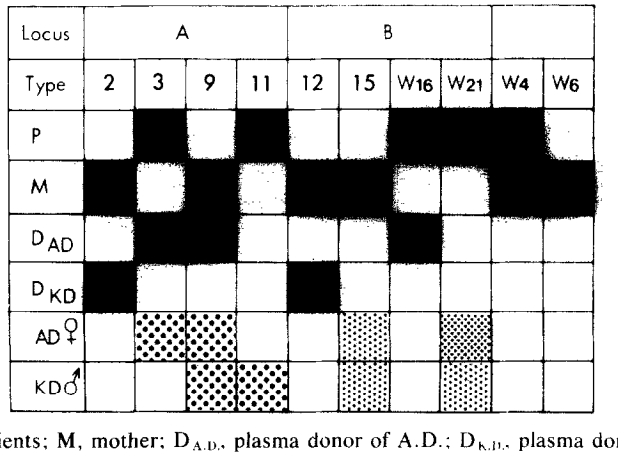
HLA-A9 and A-11 persisted in K.D.'s serum. These determinants could not have been derived from the donor.

Fibroblasts. Three methods were used to investigate HLA antigens on cultured fibroblasts. Only the HLA-A antigens were detected. For the HLA-B antigens some weak reactions were demonstrated.

These studies made it possible to draw tentative HLA-A and -B genotypes

TABLE 8

HLA-A AND -B DETERMINANTS ON LYMPHOCYTES OF THE PARENTS AND THE PLASMA DONORS[a]



[a] P, father of patients; M, mother; $D_{A.D.}$, plasma donor of A.D.; $D_{K.D.}$, plasma donor of K.D. See Table 7 for explanation of symbols.

(Tables 7 and 8). It was concluded from these data that the children inherited the same haplotype from the mother (A9-B15) but different haplotypes from the father. The HLA-B antigens from the father were not reliably determined. The occurrence of BW21 in both patients could be explained by assuming either the existence of a paternal recombination or technical error.

HLA-D and HLA-DR (Allotypic B-cell) Determinants

D-Determinants. In one-way MLR with unrelated donor lymphocytes, the stimulating capacities of patients' lymphocytes were positive but moderate (40–60% of normal levels).

DR-Determinants. Two of six anti-DRw7 sera were positive with enriched B-cell suspensions of the patients. The other anti-DRw7 sera showed weak or negative reactions. The majority of the other anti-B-cell sera was negative. Removal of the monocytes by iron depletion did not alter these results.

B-2-Microglobulin

Peripheral blood cells. By immunofluorescence B2M was detected on only 40% of patient K.D.'s PBL and these cells were identified as B-cells (Fab-positive) by a double-staining technique (Table 9). The density of the fluorescent staining for B2M on B-cells was reduced. Likewise patients' monocytes, identified by phase contrast microscope, showed reduced fluorescence for B2M. The reaction of radiolabeled anti-B2M serum with mononuclear cells was half of normal. Thrombocytes did not show B2M staining. Cultured fibroblasts were positive. Of PBL, 50–60% were not stained by the anti-B2M sera. Because 95% of B2M-staining cells in PBL were shown to be B-lymphocytes (Fab-positive) it was concluded that T-lymphocytes did not have B2M on their membranes.

TABLE 9

LYMPHOCYTE (SUB)POPULATIONS AND OTHER CELLS WITH SURFACE β-2-MICROGLOBULIN[a]

	Antiserum	Patient K.D. ♂	Control Mean (ranges)	
Peripheral blood				
Mononuclear cells	B2M	36–46[b]	100	
Lymphocytes	Fab	41	17	(10–26)
	HTLA	54	81	(68–89)
Fab-positive lymphocytes	HTLA	0[c]	0[c]	
B2M-positive lymphocytes	Fab	95[d]	17	(10–26)[d]
		Density of B2M[e]		
B2M-positive lymphocytes	B2M	+	++	
Monocytes	B2M	+	++	
Thrombocytes	B2M	–	++	
Cultured fibroblasts	B2M	+	++	

[a] Values expressed as numbers of positive cells/100 cells in isolated cell suspensions.
[b] Results of two investigations.
[c] Percentage of Fab-positive lymphocytes, which also stained with α-HTLA.
[d] Percentage of B2M positive cells, also staining with α-Fab.
[e] ++, Normal density per cell; +, lowered density per cell; –, no reaction.

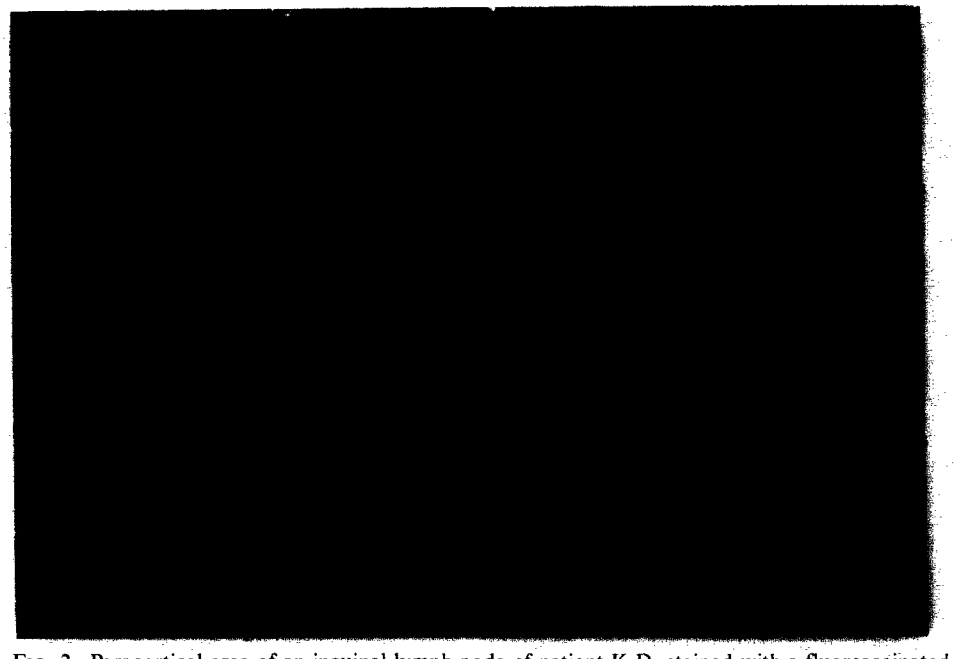


FIG. 2. Paracortical area of an inguinal lymph node of patient K.D. stained with a fluoresceinated anti-β-2-microglobulin serum. ×160.

Immunohistopathology of B2M. With the use of two different anti-B2M antisera, a clearly positive honeycomb pattern of fluorescence was seen in the thymus, spleen, intestinal wall, and lymph nodes (Fig. 2 and Table 3). Compared with tissues from other patients, the intensity of the fluorescent staining per cell appeared to be normal. Lymphoid cells were positive for B2M, as were epithelial cells and to a lesser degree endothelial cells. T-cells in the thymus, spleen, and lymph nodes were shown to carry B2M.

B2M on T-lymphocytes. There was a clear discrepancy between B2M staining on T-lymphocytes whether in tissues or in cell suspensions; the former were positive, while the latter were negative. When patient K.D.'s PBL suspensions were immersed in gelatin and the cells were further processed as tissue, all the mononuclear cells stained positive for B2M. Viable cell suspensions of K.D.'s PBL investigated in parallel showed only 40% of the cells positive (Table 9). Furthermore, the lower density of B2M on PBL membranes was not seen when PBL were processed as tissue specimen. It was concluded that T-lymphocytes either failed to express B2M on their membranes or lost B2M as soon as it appeared at the cell surface. At the same time T-lymphocytes contained B2M in or at the inner side of the membrane, only visible when cells were cross-sectioned.

Additional Data

Thymus-dependent serum factor (SF) was hardly detectable (1.5–2.7 pmol cAMP per 10^7 thymocytes; normal values 35 ± 10 pmol) (34). The erythrocytes of both patients had high levels of the enzymes adenosine deaminase and purine

nucleoside phosphorylase. Both children had normal karyograms. Chimerism in K.D.'s peripheral blood was excluded by investigation of 10^3 blasts. Glyoxalase was detected (phenotype 2-1) as well as phosphoglucomutase-3. Patient K.D.'s serum inhibited three anti-Chido and two anti-Rogers sera, which implied that he possessed these blood group antigens.

DISCUSSION

Two Turkish siblings with a history of multiple pyogenic infections and persistent candidiasis had a severe hypo-γ-globulinemia. One patient (A.D.) produced IgM in sufficient amounts, the other (K.D.) had a low level. This was reflected in the numbers of IgM plasma cells found in bone marrow biopsies. Despite the presence of B-lymphocytes carrying immunoglobulins of all classes, both patients showed a failure of B-cell differentiation to IgG- and IgA- containing cells. This might indicate a disturbance in the switch from IgM to IgG and IgA production (2). This particular B-cell differentiation phase is considered to be induced by antigenic stimulation and to be mediated by T-cells (3). There was no defect in the early stages of T-cell differentiation (stem cell → T-precursor → thymus-lymphocyte) as can be concluded from positive *in vitro* proliferative responses to lectins and the relatively normal percentages of E-rosette-forming cells and $HTLA^+$ cells. The grossly normal thymus histology also excluded severe T-cell defects. The absence of thymus-derived serum factor might indicate deficient maturation of T-cells from the intrathymic stage on. The low absolute numbers of T-lymphocytes also indicated insufficient maturation. Furthermore, defective T-cell function was shown by the lack of lymphocyte proliferation after antigenic stimulation. Lack of T-cell dependent B-cell maturation, induced by PWM stimulation, does not distinguish between a T-cell function defect or an intrinsic B-cell defect.

The final diagnosis was partial combined immunodeficiency, characterized by severe hypo-γ-globulinemia with B-cells present and partial T-cell deficiency, mainly expressed at the level of post-thymic functions. Immunogenetic studies revealed a lack of HLA-A and -B determinants on lymphocytes. A structural genetic defect in the HLA region on chromosome 6 was unlikely for the following reasons: Gross defects in chromosomes 6 in the area of the HLA loci were excluded by normal karyograms and by the presence of various gene products coded in that area (complement components: C2, C4, Factor B; enzymes: phosphoglucomutase-3, glyoxalase; and blood groups: Chido, Rogers); selective defects within the HLA region appeared to be excluded by the presence of HLA-A and -B determinants on platelets, on lymphoblasts, and especially in serum and on cultured fibroblasts. The studies on lymphoblasts were hampered by technical difficulties. The lymphocyte-dependent cytotoxicity assay was unreliable due to the possibility of additional specificities in the HLA antisera used. The CML assay, especially the lytic phase in this assay, is supposed to be mediated by HLA-A and -B determinants on the target lymphoblasts but does not exclude a role for other target cell determinants. However, a lymphoblast cell line derived from patient K.D.'s blood lymphocytes also expressed the predicted HLA-A and -B determinants (37). The studies on the D- and DR-determinants were inconclusive. The intermediate stimulatory capacity did not prove the presence of the

D-locus determinants, because theoretically this might have been mediated by determinants coded by minor D-loci within or outside the HLA region. Because only two of six well-characterized HLA-DRw7 antisera showed clearly positive reactions, it could not be concluded that patients had the HLA-DRw7 determinant. In conclusion, the immunogenetic studies showed a lack of HLA-A and -B determinants on lymphocytes, which appeared not to be caused by a structural gene defect.

Lack of HLA-A and -B determinants on lymphocytes has been described in two other cases. One was a patient with Hodgkin's disease, in which the "loss" of HLA determinants was transient (38). The other, an Algerian infant, was strikingly similar to the Turkish siblings (8). He had partial combined immunodeficiency, characterized by severe antibody deficiency, with B-lymphocytes, normal mitogenic response of lymphocytes, and a low absolute T-cell number. There was an absence of HLA-A and -B antigens on lymphocytes but they were present in serum. The parents were consanguineous. HLA typing data of the family showed one healthy sibling with the same genotype as the patient, which excluded linkage of either the immunodeficiency or the lack of HLA determinants with a particular HLA genotype.

Although there was no known parental consanguinity in the Turkish family, the coincidence of two siblings of different sex in one family from an isolated Turkish village, argues for an autosomal recessive genetic trait.

The association of lack of HLA determinants with similar lymphocyte differentiation defects in patients from two separate families suggests a causal relationship between HLA determinants expression and normal immune function. In view of the existing knowledge (4, 5) it is tempting to speculate that an autosomal genetic trait leads to an HLA determinants expression defect, which in its turn leads to a lymphocyte differentiation disorder.

The studies of B2M may indicate how the genetic defect is exposed by the absence of HLA-A and -B determinants. B2M is required for the expression of HLA-A and -B determinants on the surface of lymphocytes, as has been shown by hybridization experiments with Daudi cells (39). The finding of B2M without detectable HLA determinants on patients' B-lymphocytes argues against a defect in HLA antigen expression by mere absence of B2M. However, the fact remains that T-cells did not express B2M on their surface, although they appeared to contain B2M in the membrane. This might be explained by postulating either an immunologically undetectable structural defect in B2M or a membrane defect, in both cases leading to defective "anchorage" of B2M on the T-cell membrane. This anchorage defect would then cause rapid loss of B2M from the membrane and defective fixation of HLA determinants, which is normally accomplished in cooperation with B2M (40). The presence of B2M in patients' sera suggested that B2M reached the surface but actually did not imbed in the lymphocyte membrane. Although B-cells appeared to express reduced amounts of B2M on their surface, it is difficult to explain why there was a difference between B- and T-cells other than by assuming that there is a difference in B2M anchorage and/or B2M-mediated HLA determinant fixation on B- and T-cells. Also cell membrane turnover might be different. That cell turnover disorders can lead to loss of HLA determinants, has been shown in fibroblast cultures from patients with progeria (41). Normal

expression of other cell membrane markers such as Ig on B-lymphocytes, E-receptors on T-lymphocytes, and the Fc and complement receptors suggests that any membrane defect, if present, is selective.

In conclusion, the described association of a partial combined immunodeficiency, absence of HLA-A and -B determinants on lymphocytes, and a loss of B2M from T-lymphocytes, indicates an interrelationship between lymphocyte differentiation and HLA determinants and B2M on lymphocyte membranes.

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