THE INFLUENCE OF HLA CLASS II ANTIBODIES ON HTLV-III ("AIDS") ANTIBODY ELISA RESULTS

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Epidemiological data suggest, that the human T-cell-lymphotropic virus (HTLV-III) ist the causative agent of the acquired immune deficiency syndrome (AIDS). Based on virus antigen preparations of the infected leukemic H9 cell line, commercial HTLV-III ELISA tests have become available a few months ago. Screening for HTLV-III antibodies at every blood donation will probably become mandatory in the Federal Republic of Germany (FRG) after October 1, 1985 and is already now performed routinely in some blood banks. The number of reported AIDS cases is rapidly increasing in many European countries; with a rate of 6.6 per million population, Denmark and Belgium are the two countries with the highest rates (Table 1, MMWR 34/11, 147-150, 1985, CDC, Atlanta, USA).

TABLE 1. Reported acquired immunodeficiency syndrome cases and estimated rates per million population — 17 European countries

Country	Oct. 1983	July 1984	Oct. 1984	Dec. 1984	Rates
Austria	7	0	0	13	1.7
Belgium	38	0	0	65	6.6
Czechoslovakia	0	0	0	0	0.0
Denmark	13	28	31	34	6.6
Finland	0	0	4	5	1.0
France	94	180	221	260	4.8
Federal Republic of Germany	42	79	110	135	2.2
Greece	0	2	2	6	0 6
Iceland	0	0	0	0	0.0
Italy	3	8	10	14	0.3
Netherlands	12	21	26	42	2.9
Norway	0	0	4	5	1.2
Poland	0	0	0	0	0.0
Spain	6	14	18	18	0.5
Sweden	4	7	12	16	1.9
Switzerland	17	28	33	41	6.3
United Kingdom	24	54	88	108	1.9
Total	**260**	**421**	**559**	**762**	**2.0**

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As of December 1984, a number of 135 AIDS cases has been officially registered in the FRG; a number which has now increased to 180.

HTLV-III virus isolation studies have shown the infective agent in blood, semen and saliva of HTLV-III antibody positive (symptomatic and asymptomatic) individuals, exceptionally also from sero negative individuals. The modes of transmission are shown in table 2:

HTLV-III VIRUS ISOLATION STUDIES

HTLV-III has been isolated from : blood
semen
saliva
of HTLV-III antibody positive (symptomatic and asymptomatic) individuals, exceptionally also from seronegative individuals

MODES OF TRANSMISSION

1. Intimate sexual contact
2. Sharing of contaminated needles, razors, toothbrushes
3. Transfusion of whole blood
   blood cell components
   plasma
   clotting factor concentrate (not heat-treated)
   (immunoglobulin, albumin, plasma protein fraction and hepatitis B vaccine are not involved so far
4. Transplantation of body organs
5. Sperm (cryo-preserved)
6. Mother to child at birth

Besides these routes of transmission, the question of a new, possible occupational hazard for health care workers (clinical, laboratory and allied professionals) has been discussed in the USA (MMWR 32, 450-452, 1983). The principles for preventing AIDS transmission in persons performing necropsies or providing mortician's services are shown in table 3:

PRINCIPLES FOR PREVENTING AIDS TRANSMISSION IN HEALTH-CARE WORKERS AND ALLIED PROFESSIONALS

Persons performing necropsies or providing morticians services:

1. As part of immediate post-mortem care, deceased persons should be identified as belonging to one of the above three groups (AIDS, ARC, individuals with epidemiologic risk) and identification should remain with the body
2. The procedures followed before, during and after the postmortem examination are similar to those for hepatitis B. Personnel involved in performing an autopsy should wear double gloves, masks, protective eyewear, gowns, waterproof aprons and shoe coverings. Contaminated instruments and surfaces should be handled as infective
3. Appropriate precautions to prevent parenteral or mucous-membrane exposure of personnel to body fluids should be evaluated

The biosafety guidelines for use of HTLV-III and related viruses mention the accidental parenteral self-inoculation, droplet exposure to mucous membranes by splashing or spraying of infectious materials (possibly also aerosols), contact exposure of broken skin as well as pricking, puncturing, cutting of skin with scalpels or other sharp objects like broken glassware. In view of the epidemiological situation arising in Europe and the quality of the different commercial ELISA-techniques, the question of sensitivity and specificity of those tests for the reliable identification of infectuous materials is of importance from different forensic-serological aspects.

After completion of a HTLV-III antibody screening pilot study in German blood donors (Seidl and Kühnl, 1985), we were interested in possible causes for false-positive reactions observed in HTLV-III antibody ELISA-tests. In particular the question, if HLA antibodies might cause such reactions was investigated. A survey of HTLV-III antibody tests on 33.603 German blood donors correlated to confirmatory tests (Kühnl et al., 1985) has shown a marked discrepancy between the primary ELISA screening proce-

dures (0 to 2.91 % in different German regions) and 0.07 to 0.40 % in the confirmatory tests: Western blot (WB), immunofluorescence assay (IFA) or radio-immunoprecipitation assay (RIPA).

We tested 248 HLA ABC-antisera directed against all common HLA-specificities as shown from our experience with local serum sets, Eurotransplant (ET) and Collaborative Transplant Study (CTS) trays.

In addition, 336 HLA DR-antisera (ET DR'85, CTS, local FM DR'85 set and 9. IHWS DR antisera set including MCA) were tested by three different HTLV-III antibody ELISA's (Abbott, batch # 74089 HR, 75207 HR; ENI (Viramed) # 2362 and Organon # 850307). The results are shown at table 4:

RESULTS

POSITIVE HTLV-III ANTIBODY EIA RESULTS WERE OBTAINED WITH

0/248 HLA ABC ANTISERA

9/336 HLA DR ANTISERA; SPECIFICITY *

ANTI-DR4	(7)
ANTI-DR4+7	(1)
ANTI-DR4+7+9+ DQW3	(1)
ANTI-DR7	(1) **

* 9/27 tested DR4 antisera gave positive results

** weak positive

Apparently the DR4 specificity is present on the HTLV-III infected H9 cell line in homozygous or heterozygous state. Alternatively, a loss of the second DR antigen on the leukemic H9 cells may be discussed. We assume, that HTLV-III incorporates the DR4 molecules from the cell surface during the process of "budding". DR4 may than be "harvested" together with the virus from the cell culture and be present as a contaminant in the HTLV-III antigen preparation used for the ELISA test (both Abbott and ENI). It remains unclear, why the T-cell-derived-leukemic H9 cell

expresses the "B-cell-specific" DR molecules. A depression of an operator gene by conversion of a "normal" into a leukemic T-cell may be one of the mechanisms, which is responsible for the presence of DR on a T-cell. The fact, that only 9 of 27 (= 33 %) DR antisera tested, reacted with the ELISA antigen preparation might be explained by

a) a partial destruction of the DR4 epitope on the native DR4β-chain by ultrasonication and detergent treatment of HTLV-III infected cell culture supernatants
b) the expression of HLA-like structures on human tumor cells, as this has been reported for the prostate adenocarcinoma PC93 (Claas and Steenbrugge, 1982).

The question, why there is no obvious reaction with HLA class I antigens (ABC) remains unsolved. The complete HLA pattern of the H9 cell line has not yet determined by standard microlymphocytotoxicity assays to our knowledge. Monoclonal antibodies, directed against DR4 (also together with other HLA specificities) gave no reactions in the HTLV-III ELISAs. They are not likely to react with the goat AHG (Coombs serum) used as a conjugate in the ELISA, since these antibodies were produced in mice.

In this context it is remarkable, that the main target of the HTLV-III attack, the T4 (helper/inducer) lymphocytes recognize their antigen MHC-class II-(= DR,DQ,DP)-restricted, whereas T8 (suppressor/killer) cells cooperate in connection with MHC-class I (HLA ABC) molecules as restriction elements. An interaction of the T4 antigen of T-cells, and the class II MHC antigen of the antigen-presenting cell (APC = macrophage) appears possible; this interaction would mean another positive signal besides the antigenic stimulation of the T-cell receptor. We may speculate if the T4 antigen, which is apparently the "crucial" anchorage for later HTLV-III incorporation in the cell, and HLA DR molecules are also involved in the "budding" of the virus from the cell surface.

The extreme polymorphism of the HTLV-III envelope-proteins (env-gene) may thus reflect a response to the extreme human polymorphism of the MHC and also of a possible genetically determined T4 heterogeneity, which influences the clinical course of an individual after HTLV-III-infection.

The possible implications of false-positive HTLV-III antibody results caused by anti-DR4 are summarized in table 5:

POSSIBLE IMPLICATIONS OF FALSE POSITIVE HTLV-III ANTIBODY RESULTS CAUSED BY ANTI DR4:

1. Homosexuals immunized by sperm and WBC via rectal mucosa
2. I.v. drug addicts sharing contaminated needles
3. Hemophiliacs under FVIII concentrate substitution therapy
4. Hemodialysis patients (often polytransfused) in view of scheduled transplantations and immunosuppressive therapy
5. Women after pregnancies with development of HLA antibodies, which may be rejected as blood donors. Confirmatory tests like Western blot, immunofluorescence assay or radio-immunoprecipitation may be inconclusive at low HTLV-III antibody levels observed in early stages of HTLV-III infection

It is obvious, that lacking specificity of HTLV-III assays may cause severe problems in the interpretation of the restuls. In particular, HTLV-III positive hemodialysis patients may be excluded from transplantation and the immunosuppressive therapy requested thereafter. Confirmatory tests (WB, IFA, RIPA) cannot resolve this problem, since they are based on antibody detection as well and low antibody levels can be expected in immunocompromised hemodialysis patients (e.g. also after HBsAg vaccination).

In women, the development of HLA DR antibodies may lead to their exclusion as blood donors after pregnancies with HLA DR antibodies. It is also conceivable, that positive test results obtained with this first generation of HTLV-III antibody ELISAs are observed in individuals belonging to the known AIDS risk groups

(homosexuals, i.v.-drug addicts and hemophiliacs). The negative outcome of subsequent confirmatory tests again may not be conclusive in cases with low antibody concentrations.

In conclusion, we have shown HLA-DR4 antibodies as one possible cause for false-positive HTLV-III antibody ELISA restuls, since the probability, that all nine different donors of the DR4 antisera were HTLV-III infected, is very small.

The sensitivity and specificity of the commercially available tests (Abbott, ENI, Organon) is satisfactory in general, as we may judge from our routine blood donor screening (n = 20.000, Hessen, FRG).

Of course the detection of DR4 antibodies in individuals belonging to the known risk groups does not exclude a true HTLV-III infection. Absorption experiments with DR4-positive cells led to a variable decrease of the absorption values measured in the HTLV-III antibody ELISAs in homosexuals and hemophiliacs. These preliminary data may indicate, that both HTLV-III and DR4 antibodies occur in homosexuals HLA ABC DR-immunized by sperms and white blood cells as well as in polytransfused patients.

In forensic serology, the question of an incidental self-inoculation occuring in the laboratory or at postmortem work with possibly infective materials is of some importance.

It should be mentioned, that hitherto no AIDS cases have been reported after simple incidental needlestick injuries of laboratory personal. One case of a "microinjection" of freshly drawn venous blood in a British nurse may rather be looked at as a "transfusion-associated" (TA) case of AIDS, a category which accounts for approximately one percent of all AIDS cases.

1. Update: Acquired Immunodeficiency Syndrome- Europe, MMWR 34/11, 147-150, 1985

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II. Proteins and Enzymes