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Erythrocyte Antibodies in AIDS Are Associated with Mycobacteriosis and Hypergammaglobulinemia

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Summary. Positive direct antiglobulin results prior to transfusion in some of our AIDS patients, as well as some reports in the literature on red cell antibodies in AIDS patients, prompted us to investigate the prevalence of erythrocyte antibodies in AIDS patients with transfusion requiring anemia. In addition we studied the question of relevant correlations with clinical diagnosis and with hematological and immunological laboratory parameters.

Of 145 consecutive hospitalized AIDS patients (CDC criteria), 34 (23%) presented with anemia requiring transfusion. With each cross-match a routine antibody screening was performed. In cases of positive reaction additional antibody differentiation was done. Diagnoses, hematologic parameters, and therapy were studied retrospectively.

Agglutination was positive in at least one test for 41% (14/34) (group 1). C3d, anti I, cold agglutinins, and IgG occurred most frequently ($n=9/8/7/6$ out of 14). Seventyfive per cent (12/14) had leukopenia ($<4000/\mu\text{l}$), 57% (8/14) had thrombocytopenia ($<150000/\mu\text{l}$), and 43% (6/14) showed both. Average values for leukocytes, thrombocytes, and CD4-positive lymphocytes did not differ significantly in patients with (group 1) and without (group 2) erythrocyte antibodies. Average gamma globulin levels were significantly increased in group 1 (23.2 g/l versus 16.9 g/l; $p<0.001$).

In group 1, 64% (9/14) had proven mycobacteriosis (6 atypical), in contrast to only 15% (3/20) in group 2 ($p<0.05$). There were no significant differences between the two groups in prevalence of

other opportunistic infections, malignant lymphoma, and Kaposi's sarcoma.

Autoimmune versus infectious pathogenesis of pathologic erythrocyte antibodies in AIDS has been discussed in the literature. Our results show an association of mycobacteriosis with positive direct antiglobulin test, suggesting that autoimmune features might be triggered by certain pathogens.

Key words: Erythrocyte antibody – Direct antiglobulin test – Coombs' test – AIDS – Transfusion – Mycobacteriosis

Mild to profound anemia is seen in almost all AIDS patients with opportunistic infections. Anemia requiring transfusion is a common problem not only in patients receiving azidothymidine, but also in patients with severe infections. An abnormal positive direct antiglobulin test (DAT) has been observed in AIDS patients [6, 9, 11] as well as in asymptomatic HIV-infected patients [9]. It is not thought to contribute to significant clinical hemolysis and morbidity. Yet, life-threatening hemolytic complications can occur [9, 13].

Antibodies bound to the red cell membrane were detected in AIDS patients as early as 1981. Toy and colleagues reviewed the transfusion service record at San Francisco General Hospital between 1981 and 1983 and found a prevalence of 18 percent [11]. DAT results were positive in 10/55 pretransfusion tests, which is a strikingly high prevalence compared with 0.6% positive DAT in the general hospital population [11]. Other reports confirmed this high prevalence, and figures range

Abbreviations: ab=antibodies; AIDS=acquired immunodeficiency syndrome; ARC=AIDS-related complex; CD=cluster of differentiation; CDC=centers for disease control; CMV=cytomegalovirus; DAT=direct antiglobulin test; HIV=human immunodeficiency virus; KS=Kaposi's sarcoma; NHL=non-Hodgkin's lymphoma; PcP=Pneumocystis carinii pneumonia

even higher, from 21 to 43 percent, in patients with AIDS [6, 8, 15].

Some reports hint at an association of DAT with other hematological abnormalities such as leukopenia, lymphopenia, and immune thrombocytopenia [4, 6, 11, 15].

The first objective of our study was to compare the prevalence of erythrocyte – bound antibodies reported in the literature with our population of AIDS patients for such factors as geography.

Secondly, we investigated whether diagnosis and laboratory parameters (hematologic, immunologic) differ significantly in patients with and without erythrocyte antibodies.

Patients and Methods

Of 145 consecutive AIDS patients (diagnosis according to the criteria defined by the CDC) seen either at our outpatient clinic or admitted to our hospital, the 34 who presented between 1983 and 1988 with anemia requiring transfusion were retrospectively. All 34 patients were evaluated homo- or bisexual and seropositive for anti HIV-1 in ELISA and Western Blot. Only one patient had received prior blood transfusions.

Cross-matches were done in NaCl-, Bromelin-, and LISS-Coombs phase, with a control with the patient's own erythrocytes and serum in the LISS-Coombs phase. With every cross-match a screening for erythrocyte antibodies was performed. If any of these tests results were positive, a further antibody differentiation was done with panels of 8 to 10 sorts of erythrocytes and/or with specific antisera for C3, C3d, C4, IgG, IgA, IgM, and IgD. All reagents used are commercially available in West Germany and licensed by the Food and Drug Administration of the United States.

The 34 patients tested were divided into two groups according to the result of the direct antiglobulin test: patients with positive erythrocyte antibodies, group 1, comprised patients with a positive agglutination test (reacting with at least one of the tested antibodies), whereas group 2 consisted of patients with all tests negative.

Clinical diagnoses of AIDS had been confirmed either microbiologically or by histological examination. Hematological parameters (leukocyte and thrombocyte counts), CD4-cell counts, and gamma globulin levels were taken from patients' records.

Statistical evaluation concerning the occurrence of opportunistic infections was performed by χ^2 test. Comparison of laboratory parameters was performed using a non-parametric statistical test (U-test, Mann-Whitney-Wilcoxon).

Table 1. Types of erythrocyte antibodies in 14 AIDS patients with anemia requiring transfusion. In addition to the most frequently occurring antibodies listed here, one patient was positive for anti Le^a and another for anti Jk^b

| Patient No. | direct Coombs | C3 | C3d | C4 | Anti I | IgG | Cold Agglutinins |
|-------------|---------------|----|-----|----|--------|-----|------------------|
| 1 | + | + | + | + | — | — | + |
| 2 | + | — | + | — | — | + | — |
| 3 | — | — | + | — | — | + | — |
| 4 | + | — | + | — | + | + | + |
| 5 | + | — | + | — | + | — | — |
| 6 | — | — | — | — | — | — | + |
| 7 | + | + | + | + | + | — | + |
| 8 | — | — | — | — | + | — | — |
| 9 | + | — | + | — | — | — | + |
| 10 | + | — | + | — | — | — | + |
| 11 | + | — | + | — | + | + | + |
| 12 | + | + | + | — | — | + | — |
| 13 | — | — | — | — | + | — | + |
| 14 | + | — | + | — | — | + | — |
| Total | 10 | 3 | 11 | 2 | 6 | 6 | 8 |

Results

In 14/34 (41%) of all patients tested, erythrocyte antibodies were detectable in at least one test (Table 1). Direct Coombs testing was positive in 10 of these 14 patients. C3 was found positive in 3/14, C3d in 11 of 14, C4 in 2, anti I in 6, IgG in 6, and cold agglutinins in 8 of these 14 patients. A total of 12 patients showed a positive result in more than one test. Antibodies of the type anti Le^a and anti Jk^b were each found in one of 16 positive patients. One had had a transfusion 5 months earlier. Transfusion history was unknown in the one found positive for anti Jk^b.

Concomitant hematological abnormalities were present in both groups. In group 1 leukopenia was slightly more pronounced than in group 2, with an average of 3200/ μ l versus 3972/ μ l, but not statistically significantly. Thrombocytopenia was more marked in group 1 than in group 2 (166000/ μ l versus 192000/ μ l). Signs of overt hemolysis were found in only two patients of group 1. Group 1 had an average absolute CD4-lymphocyte count of 62/ μ l as compared with 39/ μ l in group 2 (not significant). The level of gamma globulins was significantly higher in group 1 (23.2 g/l \pm 13.3 versus 16.9 g/l \pm 4.9; $p < 0.001$). Data, averages, standard deviations, and levels of significance are given in Table 2.

Clinical diagnosis. Nine of 14 (64%) of the patients with either a positive DAT or erythrocyte anti-

Table 2. Hematological, immunological, and clinical parameters of AIDS patients with anemia requiring transfusion (group 1) and without erythrocyte antibodies (group 2)

| | Group 1 <i>n</i> = 14 | Group 2 <i>n</i> = 20 | |
|-------------------------|---------------------------------|---------------------------------|------------------|
| Leukocytes | 3200/ μ l \pm 1208 | 3972/ μ l \pm 1782 | <i>n.s.</i> |
| Thrombocytes | 166000/ μ l \pm 126000 | 192000/ μ l \pm 140000 | <i>n.s.</i> |
| CD4+ lymphocytes | 61/ μ l \pm 34 | 39/ μ l \pm 26 | <i>n.s.</i> |
| Gamma globulins | 23.2 g/l \pm 13.3 | 16.9 g/l \pm 4.9 | <i>p</i> < 0.001 |
| Diagnosis ^a | No. of patients ^a | No. of patients ^a | |
| Mycobacteriosis | 9 (6 atypical) | 3 (1 atypical) | <i>p</i> < 0.05 |
| PcP (and post-PcP) | 6 | 10 | <i>n.s.</i> |
| CMV | 4 | 4 | <i>n.s.</i> |
| Toxoplasmosis, cerebral | 1 | 1 | <i>n.s.</i> |
| Malignant lymphoma | 2 | 4 | <i>n.s.</i> |
| Kaposi's sarcoma | 3 | 3 | <i>n.s.</i> |

^a The number of patients totals more than 34 because of patients with two or more diagnoses; PcP = *Pneumocystis carinii* pneumonia, CMV = CMV disease (retinitis, colitis, or encephalitis)

bodies had proven mycobacteriosis (3 had *Mycobacterium tuberculosis*; 3, *M. avium* intracellulare; 2, *M. fortuitum*; and 1, *M. xenopii*) in contrast to only 15% of DAT-negative patients (*p* < 0.05).

Other diseases, such as *Pneumocystis carinii* pneumonia, cytomegalovirus disease, cerebral toxoplasmosis, non-Hodgkin's lymphoma, and Kaposi's sarcoma, occurred with the same frequency in the two groups (Table 2).

There was no obvious difference in treatment of the groups, especially with substances known to enhance the occurrence of a positive DAT.

Discussion

Reports on antibodies against blood cells of all types include studies not only on antibodies against thrombocytes [4, 7], granulocytes [4], and lymphocytes [2], but also on red cell autoantibodies [6, 12]. A high prevalence of a positive direct antiglobulin test and pathologic erythrocyte-bound antibodies in patients with AIDS and ARC, ranging between 18 and 43 percent, is reported in the literature [8, 11, 14]. This is in accordance with the 41%

resulting in our study in the setting of transfusion in AIDS patients. This high prevalence resembles the prevalence of antierythrocyte antibodies in autoimmune disorders such as systemic lupus erythematosus, where prevalence can range from 10 to 40 percent, while hemolysis is less frequent [10]. Although there is a high prevalence of a positive DAT in AIDS patients, hemolysis rarely occurs [9, 11, 13]. In our patients only two of 14 with erythrocyte antibodies presented with overt hemolysis.

While only individuals with anemia requiring transfusion were tested, further investigation will be necessary to determine the correlation between autoantibodies and mycobacterial infections in patients with anemia not requiring transfusion or even without anemia.

In HIV-associated nephropathy there is clear evidence for geographic and racial variability. This fact prompted us to compare the prevalence found in our patients with the literature originating from North America. Our results show that there is no geographic difference in prevalence in patients with a diagnosis of AIDS according to the CDC criteria.

Lepennec and colleagues [5] found a red cell autoantibody prevalence of only 7.5% in asymptomatic HIV-infected patients. This, however, implies a variable prevalence when different stages of HIV infection are compared, e.g., lower prevalence in earlier stages. Further studies on the relation of prevalence to various clinical and pathogenetic stages of HIV infection are necessary to determine whether both erythrocyte-bound antibodies and antilymphocyte antibodies could contribute to morbidity in HIV infection.

C3d, anti I, IgG, and cold agglutinins were most frequently detected in our study. This is in accordance with the types of antibodies described in the literature, which include mainly anti I [6], anti IgG [6, 11], and complement [12]. Again, this resembles the hemolytic anemia seen in autoimmune disorders.

Patients with erythrocyte-bound antibodies had significantly higher levels of gamma globulins in our study. This result is not at all surprising and confirms what was already known from studies by Toy [12] and Lepennec [5]. The pathogenetic meaning of the association of positive DAT with high immunoglobulin levels is not fully understood. It is suggested, however, that polyclonal immunoglobulin production might result in spontaneous immunoglobulin adherence to red cell membranes. In addition, positive DAT and the presence of circulating immune complexes were found by Inada and coworkers to correlate inversely (while

sera and eluate contained no IgG antibodies against erythrocytes [3]). Perricone and colleagues give evidence for activation of complement in patients with ARC: 62% showed an impairment of classical and/or alternative complement pathway activity associated with the presence of cleavage products of C3 and a significant reduction of complement factors [7a]. Whether presence of erythrocyte-bound C3d, e.g., is paralleled by low serum levels of C3d or other complement factors merits further investigation. Inada reported deposition of circulating immune complexes on erythrocytes via C3b receptors, which resulted in a defective clearing system for immune complexes on erythrocytes and in high membrane osmotic fragility [3]. This might be one of the mechanisms resulting in positive DAT in AIDS, as in autoimmune disorders and chronic infections resulting in hypergammaglobulinemia.

Erythrocyte antibodies may be induced (without respect to HIV) by certain pharmaceuticals such as phenacetin, methyldopa, and antibiotics. Because both groups of our patients were treated with such substances in similar frequency it is not probable that the pathogenesis of erythrocyte antibodies in AIDS is triggered by drugs. In all patients found to have a positive DAT, antimycobacterial therapy was started after the test.

Another pathophysiological explanation could be found in opportunistic infections with AIDS. Patients with ARC and AIDS are subject to latent, recurrent, and severe infections due to microorganisms known to cause red blood cell membrane modifications, including the exposure of novel carbohydrate cryptantigens that can react with naturally occurring antibodies [1]. Adams et al. tested 108 patients with ARC and AIDS for the most common forms of cryptantigens and found a high prevalence of binding site for peanut lectin [1]. An association of mycobacteriosis with DAT might be explained by such mechanisms. However, there are individuals with mycobacteriosis and negative DAT. Therefore, in our opinion, the high prevalence of erythrocyte-bound antibodies in AIDS is most probably due to a set of several possible triggering mechanisms, including autoimmune and infectious causes.

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