CLIN. CHEM. 35/8, 1669–1671 (1989)

# Oligoclonal Immunoglobulins in HIV Infection

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We tested 150 patients infected with human immunodeficiency virus (HIV) for the presence of oligoclonal bands in serum, prompted by reports that these abnormal proteins may have prognostic significance. Sixty HIV-negative individuals from "at-risk" groups were tested along with 80 HIV-negative, healthy blood donors for the presence of these bands. All sera were tested by isoelectric focusing, because it is more sensitive for this purpose than more-conventional electrophoretic techniques. In the HIV-positive group, 61% of the sera had oligoclonal bands; in the HIV-negative "at-risk" group, 36% had bands. No bands were detectable in sera from the healthy blood-donor group. Some patients were also followed for differing periods throughout their infection, and changes in their oligoclonal banding patterns could not be correlated with disease progression. The fact that oligoclonal bands were found to be present without HIV infection in a substantial number of individuals from within the "at-risk" groups leads us to conclude that the presence of oligoclonal bands in HIV infection is of limited prognostic significance.

**Additional Keyphrases:** *AIDS · oligoclonal banding · isoelectric focusing*

Hypergammaglobulinemia is a common finding in HIV-infected individuals and is related to polyclonal activation of B cells after infection with HIV (*1, 2*). Some recent reports have noted the presence of abnormal immunoglobulin bands in the serum of patients with HIV infection (*3–5*). In some cases, it was suggested that these bands may have some prognostic significance and that their study may provide a biochemical marker of disease progression in HIV infection.

To investigate this possibility further, we used isoelectric focusing to screen sera from a large number of HIV-infected individuals as well as sera from individuals from "at-risk" groups but without evidence of HIV infection and also a panel of HIV-negative healthy blood donors. We used isoelectric focusing in preference to more conventional electrophoresis, because it is known to be more sensitive for the detection of minimal paraproteins and oligoclonal banding patterns (*6, 7*).

We also followed the abnormal immunoglobulin patterns seen in some individuals throughout the course of their infection, to assess whether the oligoclonal bands changed in any way that would enable their study to be of assistance in predicting any changes in the course of the disease.

## Patients and Methods

We selected a group of 150 HIV-positive individuals to include the three major "at-risk" groups—i.e., intravenous drug abusers, hemophiliacs, and male homosexuals. We also studied 60 individuals from the same groups but without HIV infection and 80 HIV-negative, healthy blood donors.

In all cases, HIV infection was diagnosed by detection of antibody by enzyme-linked immunosorbant assay (ELISA; Wellcome Diagnostics). All ELISA positives were subsequently confirmed by Western blot analysis with use of pre-prepared strips (Dupont UK, Ltd.). All control sera were similarly tested.

The isoelectric focusing technique has been described elsewhere (*7*). In brief: gels were constructed by using, per liter, 10 g of agarose (Agarose IEF, Pharmacia Fine Chemicals), 120 g of sorbitol, and 20 mL of ampholytes (pH 3–10: Pharmacia or LKB). Gels were then either fixed in a 100 g/L solution of trichloroacetic acid and stained with PAGE blue (BDH Chemicals), 2 g/L, and then destained in ethanol/acetic acid/water (35/10/55 by vol) or overlaid with specific antiserum and then washed and stained in a similar fashion.

All sera were heat-treated to 56 °C for 1 h before study.

## Results

The 150 HIV-positive sera were examined by isoelectric focusing. Oligoclonal bands were visible in 91 cases. Of the 60 HIV-negative control sera from the "at-risk" groups, 22 showed oligoclonal bands. Study of the 80 HIV-negative, healthy blood donors failed to show bands in any case. Typical bands are illustrated in Figure 1.

In all cases, oligoclonal bands were of mixed IgG kappa and IgG lambda isotypes, as was demonstrated by antibody overlay (not shown).

Table 1 summarizes these results as they apply to the different "at-risk" groups. All sera from these groups were divided into those from intravenous drug abusers, male

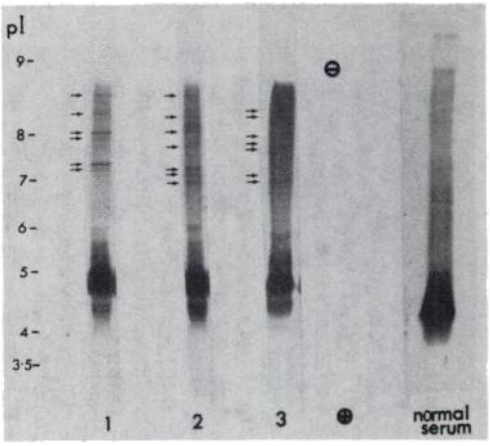


Fig. 1. Illustration of serum oligoclonal banding patterns by isoelectric focusing

Track *1*, serum from HIV-negative individual from an "at-risk" group; track *2*, serum from an asymptomatic HIV-positive subject; track *3*, serum from an AIDS patient. Also illustrated is a serum from an HIV-negative subject. Oligoclonal immunoglobulin bands are denoted by *arrows*

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Received March 28, 1989; accepted May 17, 1989.

**Table 1. Incidence of Oligoclonal Immunoglobulins in Groups "At-Risk" for HIV Infection**

| | HOMO | IVDA | HEMO | Total |
|---|---|---|---|---|
| HIV+ | 42 of 70 (60%) | 29 of 48 (64%) | 20 of 32 (62%) | 91 of 150 (61%) |
| HIV− | 8 of 20 (49%) | 5 of 20 (25%) | 9 of 20 (45%) | 22 of 60 (36%) |
| HIV− | Healthy blood donors | | | 0 of 80 |

HOMO, male homosexuals; IVDA, intravenous drug abusers; HEMO, hemophiliacs.

**Table 2. Prevalence of Oligoclonal Bands (OIB) at Different Stages of HIV Infection**

| | OIB present | OIB absent |
|---|---|---|
| Asymptomatic (n = 97) | 52 | 45 |
| Some symptoms (n = 23) | 15 | 8 |
| AIDS-related complex (n = 9) | 5 | 4 |
| AIDS (n = 21) | 18 | 3 |

homosexuals, or hemophiliacs and then further divided into HIV-positive and HIV-negative. Clearly, a large proportion of HIV-positive individuals have oligoclonal bands, and there is no significant difference between the incidences within the "at-risk" groups. It is also clear, however, that the control group containing HIV-negative individuals from the "at-risk" groups has an incidence of oligoclonal bands that far exceeds the incidence for the healthy blood-donor group.

The absence of bands in some asymptomatic patients and also patients with full-blown AIDS prompted us to examine serial samples from a number of patients longitudinally. Figure 2 shows the resulting banding patterns from four individuals. Tracks *1* to *5* are from a patient with persistent generalized lymphadenopathy, who developed AIDS-related complex over a period of two years, with very little change in banding pattern discernible. Tracks *6–9* are from a patient who remained asymptomatic for one year; they show a very strong banding pattern although the patient remained stable. Tracks *10–14* are from a patient who developed persistent generalized lymphadenopathy, and changes in banding patterns can be seen, particularly in the later samples. The waxing and waning of B-cell clones during disease progression, as evidenced by a changing banding pattern, does not allow comment on prognostic significance, because different sets of bands can be seen to change in the same patient's serum. The HIV-positive subjects were separated into broad clinical groups (Table 2), the results of which demonstrate the high prevalence of oligoclonal bands across the full clinical spectrum of HIV infection.

These studies demonstrate that there is no clear correlation between the presence or intensity of an oligoclonal immunoglobulin response and the clinical stage of HIV infection.

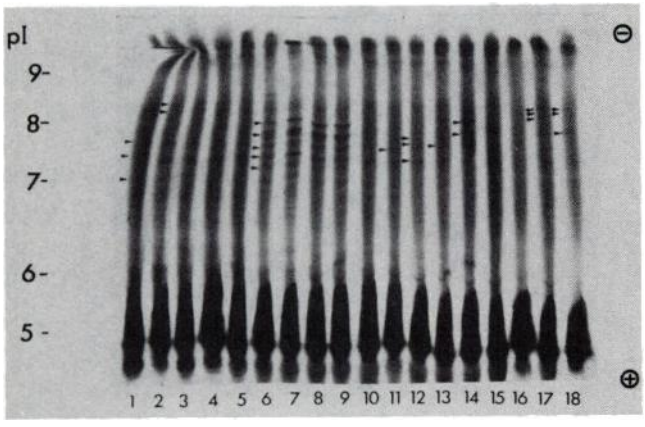


Fig. 2. Oligoclonal bands at different stages of HIV infection

Tracks *1–5*, persistent generalized lymphadenopathy/ARC 1986–1988; tracks *6–9*, asymptomatic 1987–1988; tracks *10–14*, asymptomatic/persistent generalized lymphadenopathy 1986–1988; tracks *15–18*, AIDS 1985–1988. Oligoclonal bands are denoted by *arrowheads*

## Discussion

Although we found a high incidence of abnormal oligoclonal immunoglobulins in individuals from the HIV "at-risk" groups, these bands occur *regardless* of the presence of HIV infection.

It is this last point that distinguishes this work from previous works reporting high incidences of monoclonal and oligoclonal immunoglobulins in HIV infection. Heriot et al. (*1*) reported paraproteins in eight of 15 patients with AIDS. Kouns et al. (*3*) reported that, in a review of 2500 electrophoreses, of the 19 patients with oligoclonal bands seven were HIV positive. Papadopoulous et al. (*2*) noted that the incidence of oligoclonal bands was much higher in AIDS patients with Kaposi's sarcoma (24 of 27) than in those with opportunistic infections (2 of 15). These same workers (*4*) also noted a high incidence of oligoclonal bands in symptom-free, HIV-positive individuals. Taichman et al. (*5*) suggested that the demonstration of oligoclonal banding in a patient younger than 40 years is sufficient grounds for considering testing for HIV. In none of these studies were subjects tested who were from "at-risk" groups but had no evidence of HIV infection. Our finding of a relatively high incidence (36%) of oligoclonal bands in this group suggests that a state of B-cell activation already exists in vivo, and our results cast considerable doubt on the possibility that these bands are of any prognostic significance, regardless of any observed differences within the HIV-positive groups at any stage in their infection.

Nevertheless, the mechanism by which these bands arise has yet to be discovered, and these results suggest that more than one mechanism may be involved. Their etiology may be attributable to some kind of chronic antigenic stimulation in the HIV-negative group. After HIV infection, viral antigens may have a role in their formation. However, it seems likely that more than one mechanism is responsible in the HIV-positive group, because possible etiological agents such as infections and Factor VIII injections are present before and after seroconversion. Other possibilities are the reactivation of Epstein–Barr virus-infected B cells with subsequent dysregulation and also the B-cell abnormalities that may arise secondary to the observed T-cell abnormalities in HIV-positive and -negative individuals in the "at-risk" groups. Further work on this topic is now in progress.

Although there is a difference in the incidence of oligoclonal bands between the HIV-positive and HIV-negative groups (63% and 36%, respectively), the possibility of using these bands as a diagnostic or prognostic indicator is fraught with difficulty and must be viewed with great caution.

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