October 31, 1985

Effect of Cohn Fractionation Conditions on Infectivity of the AIDS Virus

To the Editor:

Intravenous gamma globulins prepared by Cohn fractionation have been reported to transmit non-A, non-B (NANB) hepatitis viruses (1-3), suggesting that the alcohol concentrations, pH's and temperatures used in this process do not completely inactivate these lipid membrane coated viruses. As the virus causing AIDS (HTLV-III/LAV/ARV) hereinafter called the AIDS virus, may be present in plasma pools used for blood derivative manufacture (4-6), it is important to determine whether the virus can survive the fractionation procedure.

We therefore performed the following experiment: Plasma from a small number of donors shown to be free of antibody to HTLV-III by ELISA was pooled and fractionated by the Cohn ethanol fractionation procedure (7,8) in routine used at the New York Blood Center. Supernatants from Cohn fraction I containing 8% ethanol at pH 7.2, Cohn fraction II + III containing 25% ethanol at pH 7.0, and Cohn fraction III containing 17% ethanol at pH 5.4 were stored at −70° C. To aliquots of the above supernatants, which represent the potentially most virus inactivating conditions to which viruses are exposed during gamma globulin preparation 1/10th volumes of an AIDS virus stock was added. This stock consisted of RPMI-1640, 20% fetal calf serum culture supernatant from H9/HTLV-III_b producer cells (9). The virus was added after adjustment of the

supernatants to the temperature at which they are normally held during Cohn fractionation: −2°C for fraction I supernatant, −5° C for fraction II + III supernatant, and −6° C for fraction III supernatant. After 2 and 24 hours at these temperatures samples were transferred to a −8° C crushed ice/NaCl bath. These, and control mixtures of virus diluted in normal plasma, were passed through a 20 ml Sephadex G-25 column precoated with human serum albumin and equilibrated with RPMI-1640 medium. Care was taken to pass samples through these columns as rapidly as possible in order to maintain low temperatures prior to removal of alcohol. The void volumes were recovered, sterile filtered and assayed by inoculation of quadruplicate serial microtiter dilutions onto H-9 cells (10). After 14 days culture supernatants were harvested and assayed for reverse transcriptase (10). The results of this study are shown in Figure 1. Little or no AIDS virus was inactivated by the conditions used.

Due to the long storage of liquid intramuscular immune serum globulin (ISG) preparations between preparation and administration, intramuscular ISG may present little or no hazard of virus transmission, as is generally concluded. However, the increasingly popular intravenous ISG preparations are lyophilized immediately after preparation, are stored in the dry state which preserves virus infectivity, and are given in large doses intravenously. This is probably the most sensitive route for infection by hepatitis or lymphotropic viruses. Thus intravenous ISG preparations are of particular concern, as already shown for NANB transmission (3).

Our findings suggest the desirability of applying virus inactivation procedures to plasma subjected to Cohn ethanol fractionation, or to the resulting plasma derivatives, in order to sterilize them with respect not only to NANB hepatitis viruses, but also to inactivate the agent of AIDS.

Our results do not necessarily imply that intravenous gamma globulin preparations are unsafe, since some manufacturers already incorporate steps in their manufacturing procedures which have the potential for inactivating these viruses.

Furthermore, it is possible that virus may fractionate predominantly into fractions other than the gamma globulin fraction, and that any virus in this fraction could be neutralized by antibody; also it is possible that storage of intermediate fractions in the frozen state could inactivate virus.

Careful surveillance of recipients of intravenous gamma globulin preparations for development of antibodies to the AIDS virus is necessary. To date no such reports have appeared.

Alfred M. Prince, M.D.
Marcel P.J. Piët, M.D.
Bernard Horowitz, Ph.D.

The Laboratory of Virology of The Lindsley F. Kimball Research Institute, and The Blood Derivatives Program of The New York Blood Center, 310 East 67th Street, New York, New York 10021

Acknowledgments

These studies were aided by contracts No. NO1-HB-3-7009 and NO1-HB-5-7007 from the National Heart, Lung and Blood Institute. Dean Kurokawa and Lori Walakovits provided skillful technical assistance.

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