Preliminary Communication

RECOVERY AND INACTIVATION OF INFECTIOUS RETROVIRUSES ADDED TO FACTOR VIII CONCENTRATES

JAY A. LEVY GAUTAM MITRA
MILTON M. MOZEN

Cancer Research Institute, Department of Medicine, University of California, School of Medicine, San Francisco, USA, and Cutter Laboratories, Berkeley

Summary The ability of infectious retroviruses to withstand the procedures used for factor VIII concentration was investigated. Mouse retroviruses added to human plasma survived these procedures and remained infectious in lyophilised samples of factor VIII. Lyophilised material had to be heated at 68°C for several hours before substantial quantities of infectious virus became inactivated. These findings support the possible role of retroviruses in AIDS, and indicate that factor VIII concentrates must be heated to inactivate these infectious viruses.

INTRODUCTION

AT present a lymphocytopathic retrovirus is believed to be the most likely agent responsible for AIDS.[1-3] Because AIDS has occurred in haemophiliacs who have received factor VIII concentrates,[4,5] the infectious agent that causes AIDS can be assumed to be present in these preparations.

Several studies suggest hepatitis virus A, B, non-A, non-B, and the human parvovirus (SPLV) can be transmitted in factor VIII preparations.[6-9] However, enveloped viruses, such as the herpesviruses and retroviruses, might be more sensitive to the concentration process. For instance, antibodies to Epstein-Barr and cytomegalovirus are no more common in haemophiliacs receiving factor VIII concentrates than in controls.[10] Moreover, cytomegalovirus is highly sensitive to freezing and thawing and retroviruses are lysed via a complement receptor on their membrane when incubated with human plasma or serum.[11] Heating procedures have been adopted in the manufacture of factor VIII concentrates in an attempt to eliminate the possible transfer of infectious agents in these products. We examined the ability of retroviruses to withstand the procedures used for the preparation of factor VIII concentrates and we have also determined the effect of subsequent heating on virus infectivity.

MATERIALS AND METHODS

Virus.—Titres of the biologically cloned mouse xenotropic type C retrovirus recovered from a New Zealand Black mouse kidney are high (10^8 infectious particles [IP]/ml) when the virus is grown in mink lung cells.[12] A fresh preparation of this virus was mixed 1:1 with human plasma, cryoprecipitate, or factor VIII filtrates and then assayed directly for infectious virus after undergoing the procedures described below. Mouse xenotropic virus was detected by a focus assay in mink S+L− cells in which each infectious particle scores as a focus of cell transformation.[13] Virus titre was also measured by the induction of the viral core structural protein (p30) which was detected by immunofluorescence in diethylaminoethyl dextran-treated mink lung cells.[14] To increase the sensitivity of this assay, the mink cells, were passaged weekly for three weeks and then assayed for p30 antigen and the supernatants were tested for infectious virus on mink S+L− cells. The titres given represent the average of duplicate plates receiving virus at several 10-fold and at 2-fold dilutions.

Preparation of factor VIII concentrate.—The anti-haemophilic factor concentrate was purified from cryoprecipitate by refinements of the methods described by Mozen.[15] The cryoprecipitate was removed from pools of fresh frozen plasmapheresis plasma by thawing the plasma at less than 5°C, essentially according to the method of Hershgold et al.[16] The cryoprecipitate was solubilised in water and adsorbed with aluminium hydroxide to remove vitamin-D-dependent factors. This procedure was followed by acidification (pH6·3–6·6) with acetic acid, and cryoprecipitation at 5–10°C to remove fibrinogen and cold-insoluble globulins. Antihaemophilic factor present in the centrifuged supernatant was then precipitated with glycine. The precipitate was dissolved in sodium chloride, sodium citrate, and glycine, and filtered through 0·45 μm and then 0·22 μm absolute filters. 5 ml samples of the resulting filtrates were then placed into ampoules and lyophilised. Lyophilised samples were left at room temperature or 4°C or heated at 68°C for up to 96 h.

RESULTS

The virus titre (10^8 IP) was not affected by mixing with cold (5°C) plasma. In contrast, incubation of the virus with plasma at 37°C for 30 min reduced its titre 100-fold. This finding accords with the report of complement-mediated lysis of retroviruses by human serum.[11] When the virus/plasma mixture was cooled to less than 5°C, the resulting cryoprecipitate contained infectious virus which had a titre of $10^{7·2}$ IP—ie, about a 10-fold reduction in the initial titre of total infectious virus added (table I).

We next added virus to redissolved cryoprecipitate (300 ml) and found it maintained the same titre as the original virus inoculate ($10^{8·5}$IP) (table I). Subsequently, this "spiked" cryoprecipitate was subjected to the procedures used to produce factor VIII concentrates. The infectious virus titre was measured after aluminium hydroxide and acetic acid treatments as well as after glycine precipitation, resuspension in fluid, and filtration. All these procedures only reduced the total number of infectious viruses to 10^7 IP. Finally, the filtrate was lyophilised and samples, which were kept either at ambient temperature or 4°C, were tested for infectious virus. The total virus recovery was $10^{5·2}$ IP. These studies indicated a 100-fold loss in the titre of infectious virus during the lyophilisation procedure, and a 1000-fold loss for the entire concentration procedure for factor VIII. Nevertheless, a substantial amount of infectious virus remained.

In the third part of this study, we added virus to the filtrate before lyophilisation to give a final concentration of $10^{5·3}$ IP/ml. We then assayed the lyophilised sample at zero time and at time periods up to 96 h after heating (68°C). The results (table II) indicated that lyophilisation had less effect on the virus titre (which decreased to $10^{4·8}$IP/ml) than in the first study. Heating the lyophilised samples at 68°C reduced the infectious virus titre substantially in 1 h, although residual infectious virus (2 IP/ml) was still present in all three ampoules after 48 h of heating. The procedures used for virus

TABLE I—RETROVIRUS RECOVERY FROM FACTOR VIII CONCENTRATES

—	IP
I. Virus alone	10^8
Virus + plasma (5°C)	10^8
Virus in cryoprecipitate	$10^{7·2}$
II. Virus + cryoprecipitate	$10^{8·5}$
Treatment	
$Al(OH)_3$, acetic acid (pH 6·3–6·6)	$10^{7·6}$
Glycine precipitation, resuspension and filtration (0·45 μm–0·22 μm)	$10^{7·0}$
Lyophilised samples	$10^{5·2}$

IP = total infectious mouse xenotropic retrovirus particles.

TABLE II—EFFECT OF HEATING ON INFECTIVITY OF MOUSE RETROVIRUSES PRESENT IN LYOPHILISED FACTOR VIII CONCENTRATES

—	IP/ml
Virus in factor VIII filtrate	$10^{5 \cdot 3}$
Virus in lyophilised concentrates at 0 time	$10^{4 \cdot 8}$*
Heating (68°C):	
1 h	$10^{3 \cdot 3}$
12 h	$10^{3 \cdot 0}$
24 h	$10^{1 \cdot 7}$
48 h	$10^{0 \cdot 3}$
72 h	$10^{10 \cdot 2}$
96 h	NV

IP = infectious mouse xenotropic retrovirus particles.
*Figures represent average virus titre in 3–4 lyophilised factor VIII concentrates receiving this treatment. NV = no infectious virus detected.

detection included the passage of mink lung cells for over 3 weeks to permit the spread and reinfection of neighbouring cells with any infectious xenotropic virus. In these long-term experiments, virus was detected in 2 out of 3 lyophilised samples heated for 72 h, but only at a concentration of <1 il. No infectious virus was detected in the samples heated for 96 h at 68°C.

In concomitant studies, we kept a vial containing the infectious mouse virus in culture fluid at 56°C for up to 12 h and tested its infectivity. Before heating, the infectious virus content was $10^{7 \cdot 2}$ IP/ml. After heating for just 1 h, no infectious virus was detected.

DISCUSSION

A significant portion of heat-labile retroviruses which are sensitive to freezing and thawing withstand the procedures used to prepare factor VIII. Substantial inactivation was found only after the lyophilised samples of factor VIII had been heated for several hours at 68°C. These data support our earlier findings that retroviruses are resistant to freeze-drying and can be kept in a lyophilised form for at least 1 year without a decrease in titre.[17] The resistance of the lyophilised retroviruses to heat treatment contrasts with the sensitivity of these viruses in liquid phase to freezing and thawing (titre drops by 100 to 1000 fold) and to heating (completely inactivated at 56°C for 1 h). It appears that retroviruses in a lyophilised form (perhaps protected by factor VIII concentrate) are much more resistant to heat and temperature changes.

These studies suggest that if infectious retroviruses are present in human plasma at titres over 100 IP/ml, some could survive the process of factor VIII concentration and thus be administered as infectious agents to patients with haemophilia. Human retroviruses have been cultured from lymphocytes obtained from patients with AIDS,[1-3] and these viruses are also likely to be present in factor VIII concentrates. The results also suggest that the lymphocytopathic retroviruses isolated from AIDS patients might be found in plasma alone. Prolonged heating at 68°C inactivates retroviruses, and adoption of this procedure in the manufacture of factor VIII concentrates should result in materials free of these infectious viruses. The present data accord with the hypothesis that retroviruses could be the aetiological agent in AIDS.

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Correspondence should be addressed to J. A. L., Cancer Research Institute, School of Medicine, University of California, San Francisco, CA94143, USA.

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Hypothesis

AUTOIMMUNITY AND IDIOTYPES

ANNE COOKE PETER M. LYDYARD
IVAN M. ROITT

Department of Immunology, Middlesex Hospital Medical School, London

THE hypothesis that autoantibodies might be anti-idiotype antibodies to antiviral antibodies[1] stimulated a great deal of interest. We feel that it would be of value to put this hypothesis into a rather wider context of different types of cross-reactions which can give rise to autoimmunity (fig 1).

The first ideas on the induction of autoimmunity by cross-reactivity were proposed independently by Weigle[2] and Allison.[3] They envisaged the possibility that cross-reaction between an exogenous antigen and an autoantigen could provide T-cell help through the new carrier determinants, which would trigger autoreactive B cells. Examples are cross-reactions between streptococcal M protein and heart tissue in rheumatic fever,[4] and between both brain and heart tissue and *Trypanosoma cruzi* in Chagas' disease.[5] Post-rabies vaccination encephalomyelitis is thought to be due to cross-reaction with the brain material in the vaccine,[6] while Ebringer and colleagues have long championed the importance of similarities between HLA-B27 and certain strains of klebsiella in the pathogenesis of ankylosing spondylitis.[7]

We have proposed an alternative system in which the idiotype on the autoantibody may cross-react either with a

J. A. LEVY AND OTHERS: REFERENCES

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