Viral infections and IgM antoantibodies to cytoplasmic intermediate filaments

B. H. TOH, A. YILDIZ, J. SOTELO, O. OSUNG, E. J. HOLBOROW, F. KANAKOUDI* &
J. V. SMALL† *MRC Immunology, Bone & Joint Research Unit, London Hospital Medical College, London,*
* *University of Thessaloniki, Greece, and* † *Institute of Molecular Biology, Austrian Academy of Sciences, Austria*

SUMMARY

Seventy-four out of 113 sera from patients with infectious hepatitis, chickenpox, measles and mumps reacted with both smooth muscle and cytoplasmic filaments in cultured fibroblasts and neuroblastoma. Five out of eighty-five control sera also reacted in this way. That the cytoplasmic structures are intermediate filaments was suggested by their rearrangement into coils of perinuclear filaments in colchicine- or vinblastine-treated fibroblasts, but not in cytochalasin B-treated cells. The identity of these structures was confirmed by the demonstration that the same structures reacted with the post-viral sera and a rabbit and human anti-intermediate filament antibody. Immunoabsorption studies showed that twenty-seven out of thirty-two positive sera were neutralised by skeletin, the intermediate filament protein from smooth muscle. In all but one of the sera, the antibody was IgM. Antibody titres fell in the second specimen in eleven out of fourteen pairs of acute and convalescent sera. The association between viral infections and auto-antibodies suggests that production of antibody to intermediate filaments may be initiated by viruses.

INTRODUCTION

Although the current emphasis in studies of autoimmunity is on genetic mechanisms (Rose, 1978), the contribution of environmental agents is equally relevant. A role for viruses in the genesis of autoimmune disease (Editorial, *Lancet*, 1978) is a possibility that would be strongly supported if associations could be found between viral infections and auto-reactive lymphocytes or their products. In the present study, we show that an autoantibody to ubiquitous intermediate filaments (Kurki *et al.*, 1977; Gilbert, 1978) develops in children with four different viral infections, *viz.* infectious hepatitis, chickenpox, measles and mumps.

MATERIALS AND METHODS

Sera from 113 Greek paediatric patients were tested for immunofluorescent reactivity with acetone-fixed tissue cultured cells and with unfixed frozen sections of mouse stomach, rabbit liver and rat kidney. The patients, eighty-one males and thirty-two females aged between 1 and 14 years (mean 6·8 years), comprised seventeen with HBsAg-negative infectious hepatitis (Kanakoudi *et al.*, 1975), nineteen with chickenpox, thirty-eight with measles and thirty-nine with mumps. Diagnosis was based on clinical, laboratory and epidemiological data. A serum sample from all patients was taken on day 7–10 after the onset of disease and another from fourteen patients 1–2 months after recovery. Eighty-five control sera from healthy children of the same age and sex distribution were also tested.

Monolayers of cultured foetal lung fibroblasts (Toh & Hard, 1977) were examined after one to three sub-cultures. For

immunofluorescent tests, cells were sub-cultured on glass cover-slips (Chance, Propper 18 × 18 mm) in 35 mm culture dishes (Falcon 3001) containing Dulbecco's modified Eagle's medium and 5% foetal calf serum. The cells were examined after incubation for 24–72 hr at 37°C in a humidified atmosphere containing 5% CO_2/95% air. Cells were also tested after incubation with colchicine (Sigma) 0·5 μg/ml for 12 hr, vinblastine (Eli Lilly) 10 μg/ml for 4 hr, or cytochalasin B (Sigma) 10 μg/ml for 30 min. Before testing, monolayers were rinsed with warm phosphate buffered saline (PBS), fixed in absolute acetone for 5 min at −20°C and air-dried. A mouse neuroblastoma cell line (Neuro 2a, Flow Laboratories) was similarly tested.

Cultured cells were initially screened for immunofluorescent reactivity (Toh & Hard, 1977) with sera at a dilution of 1:8 in PBS. Bound immunoglobulin was detected with fluorescein-isothiocyanate (FITC) labelled sheep anti-human immunoglobulin (Wellcome). The immunoglobulin class of any positive sera was determined by tests with FITC-labelled anti-human IgG, IgA or IgM (Wellcome).

Double immunofluorescence tests were carried out to compare the reactivity of post-viral sera with a rabbit (Gordon, Busnell & Burridge, 1978) and human (Kurki *et al.*, 1977) serum of defined anti-intermediate filament specificity. The human serum was a gift from Dr P. Kurki, Helsinki University. The rabbit serum was from an animal with spontaneous smooth muscle antibody. Its immunofluorescent reactivity with cryostat sections of tissues and with cultured cells was similar to that of the human reagent. All its antibody activity was also completely inhibited by serum absorption with intermediate filament protein prepared from porcine smooth muscle. Tests were carried out by a double sandwich technique (Toh & Hard, 1977) in which human sera were traced with FITC-labelled sheep anti-human immunoglobulin and rabbit serum with rhodamine-labelled goat anti-rabbit immunoglobulin. Control experiments were carried out by comparing the staining seen with a human anti-actin antiserum (Toh *et al.*, 1976; Toh, Clarke & Ceredig, 1978) and the rabbit antiserum.

Thirty-two positive sera comprising eight each from patients with infectious hepatitis, chickenpox, measles and mumps were selected at random for immunoabsorption studies. Immunoabsorption (Toh *et al.*, 1978) was carried out with porcine skeletin, the protein subunit of smooth muscle intermediate filaments or with skeletal muscle actin or smooth muscle myosin. Intermeduate filament protein was prepared by acetic acid extraction of porcine stomach smooth muscle residue, as described by Small & Sobieszek (1977). Further purification of the filament protein was achieved by chromatography on Sepharose 6B-CL ion exchange resin in 7 M urea and employing a linear gradient of guanidine-HCl. Actin and myosin were from Dr M. Owen, National Institute of Medical Research, Mill Hill, London. The purity of proteins was established on sodium dodecyl sulphate gel electrophoresis (Small & Sobieszek, 1977).

RESULTS

Seventy-four out of 113 patients' sera stained smooth muscle of mouse stomach and blood vessels of rat kidney. In cultured fibroblasts, smooth muscle antibody (SMA) positive sera stained elaborate cytoplastic filaments (Fig. 1a). In colchicine- or vinblastine-treated cells, typical 'filament caps' (Starger & Goldman, 1977) were seen. These were perinuclear coils which occasionally extended into the peripheral cytoplasm (Table 1, Fig. 1b). By contrast, cytochalasin B treatment left the cytoplasmic filaments intact, even though the cells became extensively arborised (Fig. 1c). A perinuclear, or circumnuclear, coil of filaments was also seen in cultured neuroblastoma cells (Fig. 1d). Similar staining patterns were seen with all SMA-positive sera in neuroblastoma cells and in fibroblasts pre-treated with colchicine, vinblastine or cytochalasin B. With one exception, all the antibodies were IgM. Serum titrations with monospecific anti-IgM conjugate gave titres ranging from 1:16 to 1:128 with a mean titre of 1:64. Of eighty-five control sera tested, five gave staining patterns similar to those of test sera (Fig. 2). Falling autoantibody titres were demonstrated in eleven out of fourteen pairs of acute and convalescent sera (Fig. 3).

Double fluorochrome studies showed that our own positive human sera and the human serum provided by Dr P. Kurki with anti-intermediate filament activity stained the same structures as the rabbit antiserum (Fig. 4a, b). In no instance did one serum block staining by the other. The same result was obtained whether the human or rabbit serum was applied first. Control experiments showed that human anti-actin antibody bound to structures distinct from those of the rabbit anti-intermediate filament serum (Fig. 5a, b).

Immunoabsorption studies showed that all the antibody activity of twenty-seven out of thirty-two SMA-positive sera was neutralised by skeletin, the intermediate filament protein from smooth muscle, but not by skeletal muscle actin or smooth muscle myosin. The five positive sera not inhibited by skeletin comprised one from a patient with mumps and four from patients with infectious hepatitis.
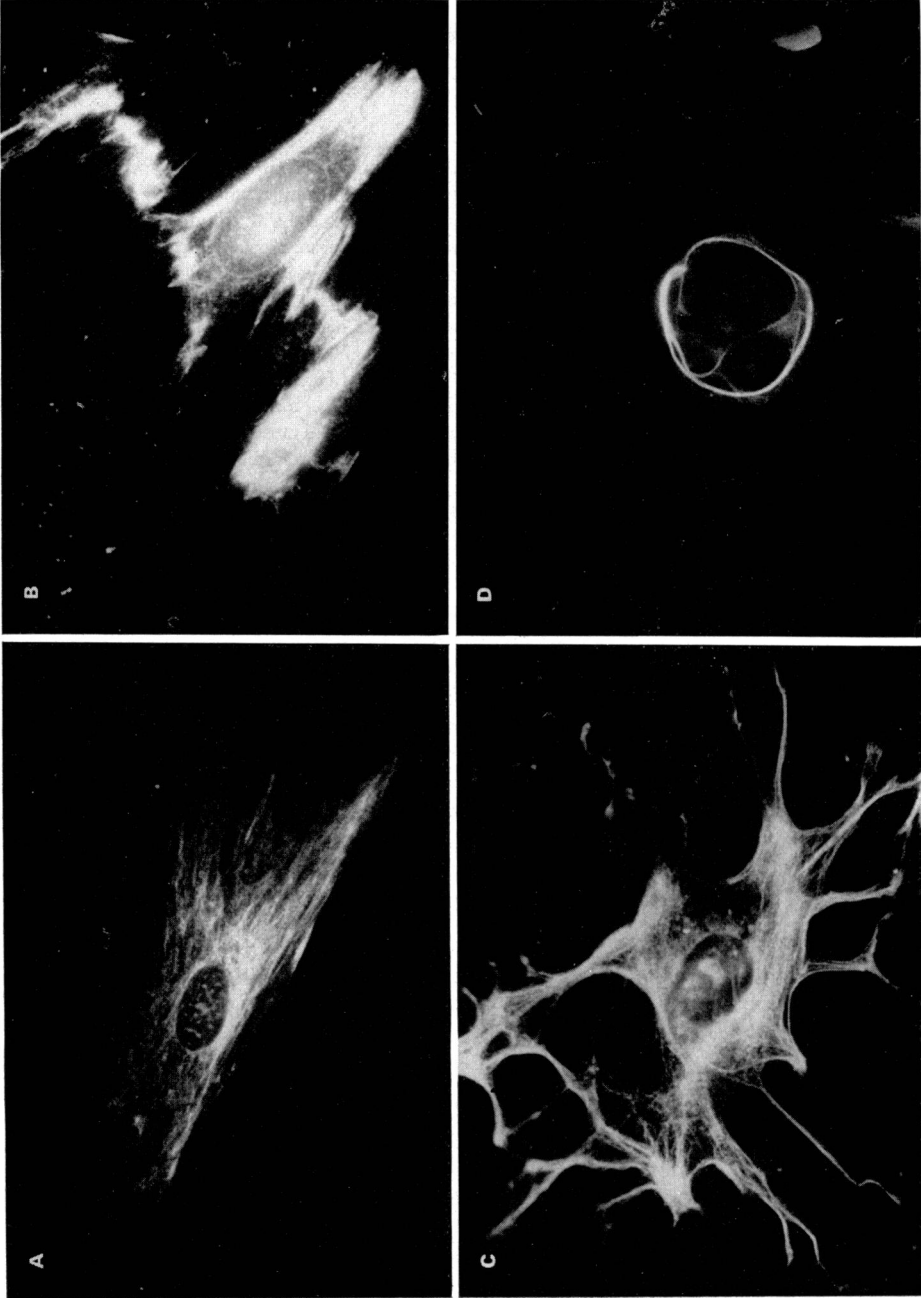
FIG. 1. Cultured cells reacted with mumps serum. Indirect immunofluorescence × 1600. Rat fibroblast showing (a) cytoplasmic filaments, (b) perinuclear filaments in vinblastine-treated cells, (c) cytoplasmic filaments in cytochalasin B-treated cells, and (d) circumnuclear filament coil in mouse neuroblastoma.

TABLE 1. Serum reactivity for intermediate
filaments in cultured fibroblasts

	Number tested	Number positive
Infectious hepatitis	17	14
Chickenpox	19	8
Measles	38	30
Mumps	39	22
Controls	85	5

DISCUSSION

Our results show that a majority of patients with four different viral infections have a common IgM autoantibody to smooth muscle, as well as cytoplasmic filaments in cultured cells. It is probable that this autoantibody is similar to the 'fibrillar anti-cellular antibody' previously reported to be associated with measles and mumps (Haire, 1972). The identification of the cellular structures as intermediate filaments was suggested by their rearrangement into perinuclear 'filament caps' (Starger & Goldman, 1977) in fibroblasts treated with microtubule-disrupting drugs, colchicine or vinblastine. One explanation for this is that microtubule disruption leads to the 'elastic recoil' of intermediate filaments because of an association between them. By contrast, the filaments remain intact after treatment with cytochalasin B, a microfilament-disrupting drug. Furthermore, the sera bind to typical filament coils in neuroblastoma cells (Jorgenson *et al.*, 1976). Double fluorochrome studies confirmed that the structures were intermediate filaments because the same structures reacted with the post-viral sera and a rabbit and human anti-intermediate filament autoantibody. The failure of human sera to block staining by rabbit serum and vice versa also suggests that some antigenic determinants on intermediate filaments reacting with human sera are distinct from those reacting with rabbit serum.

Immunoabsorption studies showed that twenty-seven out of thirty-two SMA-positive sera were neutralized by skeletin, the protein subunit of smooth muscle intermediate filaments. These observations suggest that the sera contain an autoantibody which reacts with antigenic determinants common to intermediate filaments from smooth muscle and fibroblasts. The failure to neutralize the five positive sera with skeletin suggests that these latter sera may contain an additional autoantibody which reacts
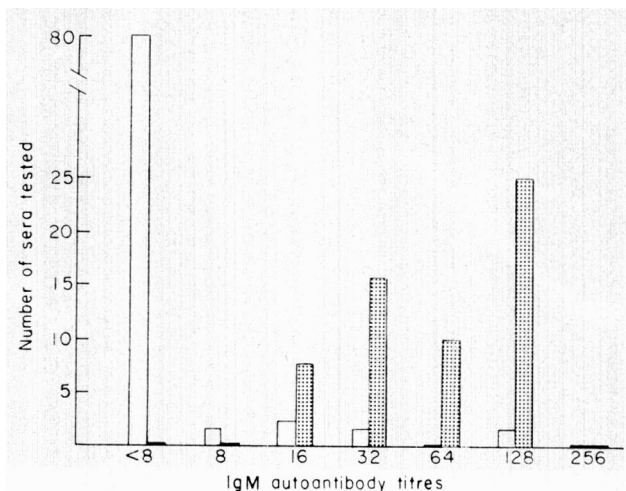

FIG. 2. Titres of IgM autoantibody to intermediate filaments in patients with viral infections (▨) and in controls (□).
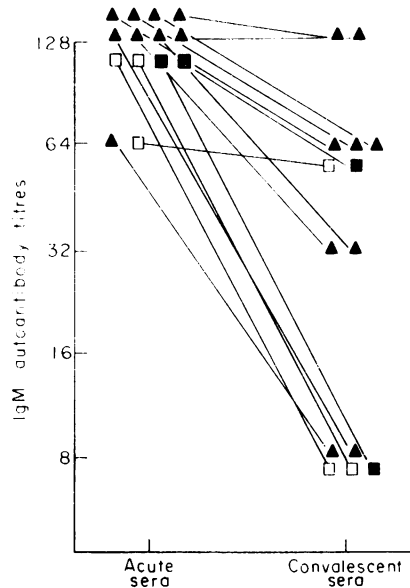
FIG. 3. Titres of IgM autoantibody to intermediate filaments in paired acute and convalescent sera from patients with mumps (▲), measles (■) and chickenpox (□).

with fibroblast intermediate filaments only. These observations are consistent with a recent report showing that intermediate filaments from fibroblasts may contain unique antigenic determinants (Franke *et al.*, 1978).

In cryostat sections of tissues, immunofluorescent staining was restricted to smooth muscle in a distribution conforming to the 'SMA-V' pattern of Bottazzo *et al.* (1976). The present study suggests that the 'SMA-V' staining pattern may chiefly reflect serum reactivity with cytoplasmic intermediate filaments. This is in contrast with the broad reacting 'SMA-T' pattern commonly associated with anti-actin antibody from patients with active chronic hepatitis.

The association of smooth muscle autoantibody and infectious hepatitis has been reported (Farrow *et*
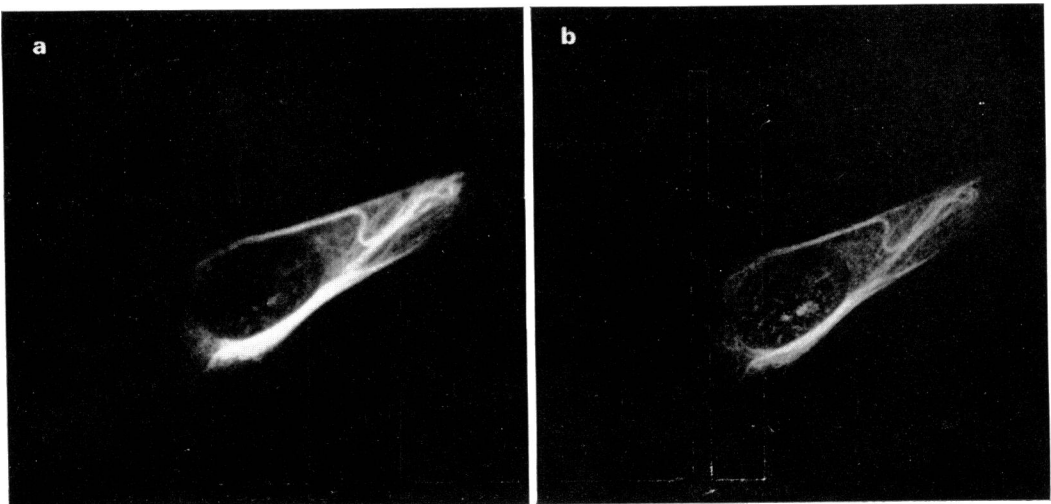

FIG. 4. The same fibroblast reacted with (a) mumps serum traced with fluorescein conjugate followed by (b) rabbit anti-intermediate filament serum traced with rhodamine conjugate. Double indirect immunofluorescence × 1600. Identical perinuclear filaments are stained.
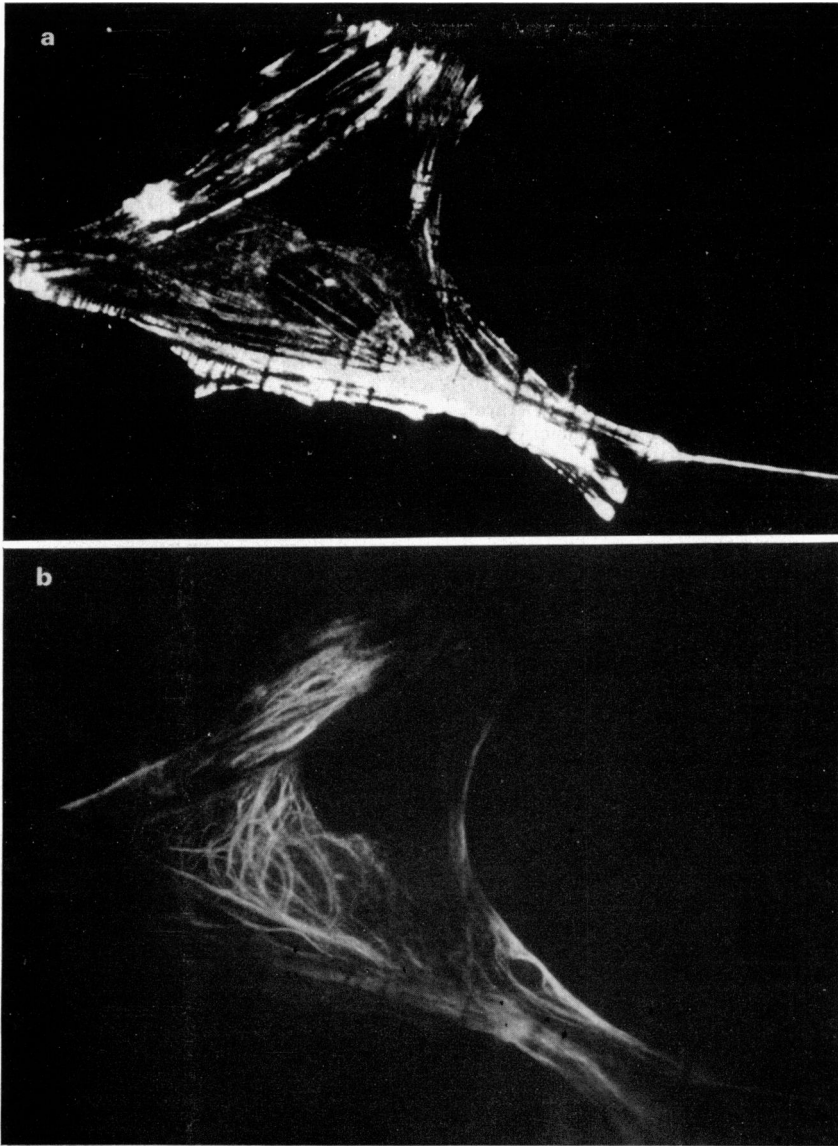
FIG. 5. The same fibroblast reacted with (a) human anti-actin antibody traced with fluorescein conjugate followed by (b) rabbit intermediate filament serum traced with rhodamine conjugate. Double indirect immunofluorescence × 1600. Different cytoplasmic filaments are stained.

al., 1970), but the present demonstration of its anti-intermediate filament specificity is interesting because smooth muscle antibody from patients with active chronic hepatitis is directed against actin (Toh *et al.*, 1978; Holborow, 1979). Another striking contrast is that while the autoantibody in infectious hepatitis is mainly IgM, the antibody in active chronic hepatitis is IgG. In this respect it resembles the smooth muscle antibody which occurs transciently in infectious mononucleosis (Holborow, Hemsted & Mead, 1973), another acute viral infection. On the basis of serum reactivity with cytoplasmic filaments in cultured cells, it should now be possible to define accurately these 'smooth muscle antibodies'.

The association between viral infections and the autoantibody to intermediate filaments suggests a causal relationship. This association is strengthened by the demonstration of falling IgM autoantibody titres in paired acute and convalescent sera. As previously suggested (Allison, Denman & Barnes, 1971)

common immunogenic units formed between viral antigen and cytoskeletal structures, such as micro-filaments and intermediate filaments, may facilitate autoimmunization by stimulating helper and/or by-passing suppressor T cells. Indeed, recent co-capping experiments have shown a close association between membrane-associated viral protein and cytoplasmic actin (Mousa *et al.*, 1978). However, other possibilities, such as a role for histocompatibility-linked immune response genes in independently promoting viral infections and autoantibody production, have not been explored.

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