

ordinary attacks of morbilli (*Lancet* 1971), and perhaps an innate factor, or a combination with exogenous influences, excites the virus and conditions it to behave in so unusual a fashion. The role of host factors, such as cell-mediated immunity, is discussed by Dr Valdimarsson below; it may be relevant that evidence has been obtained both by tissue culture and by immunofluorescence that the virus of SSPE is widely distributed throughout the body (Horta-Barbosa *et al.* 1971, Dayan & Stokes 1972), as if there were a generalized disturbance of immunity. In addition, SSPE-like illnesses have been described in rare patients with impaired immunity (Breitfeld *et al.* 1973). Immunofluorescence has made it possible to demonstrate superficial binding of IgG and β_2 to some cells that also contain measles antigens (Dayan & Stokes, unpublished observations; see Figs 1 and 2). This may be one way in which cells are damaged in SSPE, and could be relevant to possible attempts at therapy. The functional activity of chronically infected cells has barely been approached.

There has been a great increase in knowledge about SSPE in the past decade, and attention now seems to be directed towards the essential problem of the relationship between a persistent infective agent and its host – a situation of importance extending far beyond such a rare disease of the nervous system.

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Dr H Valdimarsson, Dr Gudrun Agnarsdottir and Professor P J Lachmann
(Departments of Immunology and Virology,
Royal Postgraduate Medical School,
Ducane Road, London W12 0HS)

Cellular Immunity in Subacute Sclerosing Panencephalitis

Children with agammaglobulinaemia, who cannot make antibodies, are nevertheless able to deal perfectly well with measles infection, provided their T lymphocyte function is intact. Not only are these children capable of terminating their measles infection in a normal manner, but they also develop resistance against reinfection (Good & Zak 1956). Conversely, in patients with suppressed T lymphocyte function measles is a very serious or even fatal disease which is often associated with giant cell pneumonia (Enders *et al.* 1959). Thus, although measles can be prevented by giving antibodies soon after exposure, humoral immunity does not seem to play a major role in natural resistance against the disease.

It has been suggested that after primary measles infection the virus normally persists in the body at a level which does not affect the host but is sufficient to maintain life-long immunity. Perhaps the strongest indirect evidence for this hypothesis comes from an observation which was made by Panum during a measles epidemic in the Faeroe islands in 1847. Measles had been absent from the islands for sixty years, and in the epidemic all the inhabitants caught the disease except those who had been exposed as children sixty years earlier. It is hard to believe that immunological memory can persist in this way for sixty years without any booster effect whatsoever.

If, however, after a primary measles infection, a compromise is normally established between virus and host, then a decrease in the host's resistance, a modification or a mutation of the virus itself, or possibly a combination of both, could lead to reactivation of the virus. Such variation is certainly well known, for instance in the relationship between humans and the herpes type viruses.

Dr Dayan (p 1123) has already discussed the current stage of our knowledge about the etiology and pathogenesis of subacute sclerosing panencephalitis (SSPE). It was in fact Dr Dayan who two years ago first brought our attention to this disease and the interesting questions it posed as a model for persistent viral infection in humans.

The questions to which we have addressed ourselves include: (1) Are SSPE patients immunologically abnormal and, if so, is the abnormality primary or does it merely result from the persistent virus infection? (2) If these patients are defective immunologically, is it possible to correct or stimulate their defence mechanisms and thereby halt the progress of their disease?

So far we have investigated 10 patients, 7 boys and 3 girls, ranging in age from 5 to 21 years, including one pair of siblings. The clinical diagnosis was verified in all cases by the typical EEG changes associated with very high titre of measles antibodies in serum and cerebrospinal fluid (CSF). In most of the patients measles antigens were also detected in lymphoid cells derived from CSF by immunofluorescent techniques (Dayan & Stokes 1972).

Cellular Immunity

For reasons mentioned earlier, we have focused our studies on cell-mediated (T lymphocyte dependent) immunity. The majority of our patients were anergic to common skin test antigens such as candida, streptokinase-streptodornase (SK-SD), PPD and dinitrochlorobenzene.

The anergy seemed to be more pronounced if the disease was advanced since 5 patients in a terminal stage were negative to all skin test antigens, whereas 4 patients tested early in their disease were all able to express delayed hypersensitivity (DH) to at least one antigen. Furthermore, in 2 patients previously positive skin responses became negative as the disease progressed. It is clear, however, that this generalized anergy is not due to a severe depression or depletion of T lymphocytes. Circulating T lymphocytes were always present in normal number as determined by the sheep red cell rosetting technique, and their *in vitro* reactivity to phytohaemagglutinin (PHA) was also normal. Neither does it appear to be due to a selective depression of T lymphocyte function since in the anergic patients there was a normal lympho-

cyte transformation (LT) response and MIF production *in vitro* after challenge with candida, SK-SD and PPD.

Measles virus skin test antigen, made from Schwartz strain (Glaxo), gave a weak but definitively positive DH response in 35 out of 60 control subjects. The SSPE patients were all anergic to this antigen, regardless of the stage of their disease and, in contrast to other antigens, measles antigen never stimulated their lymphocytes to transform (7 patients) or produce MIF (4 patients) *in vitro*. The measles antigen used *in vitro* induced a low but significant proliferative response of lymphocytes in approximately 50% of healthy controls. Thus even early in their disease, SSPE patients seem to be specifically unresponsive to measles virus.

Several other groups using skin tests, lymphocyte transformation and leukocyte migration techniques have reported findings in SSPE similar to ours (Lischner *et al.* 1972) but conflicting reports have also been published (Gerson & Haslam 1971, Mizutani *et al.* 1971).

Measles Cytotoxicity

Our control subjects, including both children and adults, all had a history of measles confirmed by the presence of HI measles antibodies in serum. In spite of this not more than 50% showed significant DH, LT or MIF reactivity to measles virus, and we were unable to improve this by various modifications of the measles antigen preparations. Furthermore, the responders were in most instances only moderate to borderline reactive. Partly due to these difficulties and partly because we felt that tests were needed which might reflect more directly the mechanisms responsible for containing measles infection *in vivo*, we devised a new *in vitro* method for assessing cellular immunity to measles. Briefly, this method, described in detail elsewhere (Valdimarsson, Agnarsdottir & Lachmann 1974, in preparation), is based on using measles virus which has been adapted to grow in chicken embryo fibroblasts without producing cytopathic effect. Uninfected and measles-infected fibroblasts are cultured for 24–72 hours in the presence of tritiated thymidine. After a further 24–48 hours' incubation with and without lymphoid cells, the tissue culture wells are thoroughly washed and the remaining fibroblasts dissolved in deoxycholic acid. Cytotoxic effect is determined by measuring radioactivity released from the fibroblasts remaining adherent after the washing and the effect of the lymphoid cells is expressed as a percentage by the following formula:

$$\frac{\text{CPM in presence of serum only} - \text{CPM in presence of serum and lymphocytes}}{\text{CPM in presence of serum only}} \times 100$$

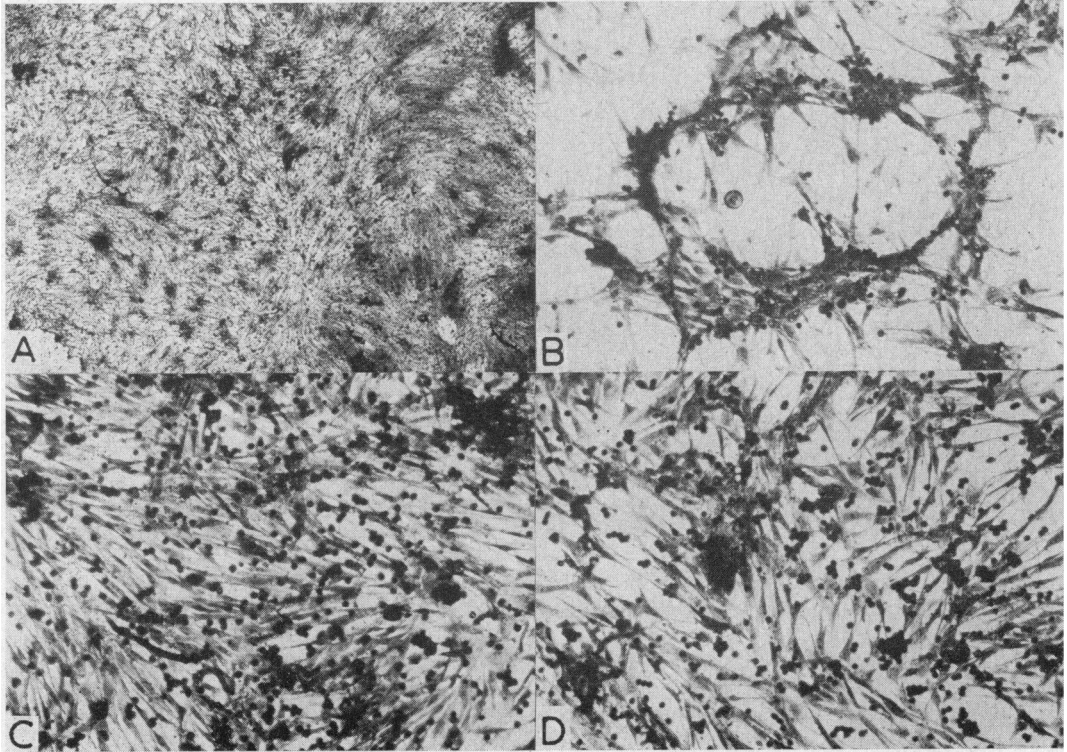


Fig 1 Chicken embryo fibroblasts after termination of a cytotoxic assay. All cultures were first incubated for 36 hours in medium containing 10% fetal calf serum, followed by a further 36 hours' incubation as indicated: **A**, measles-infected fibroblasts cultured in medium and 10% normal human serum. **B**, measles-infected fibroblasts after co-cultivation with human lymphoid cells and 10% normal human serum. **C**, uninfected control fibroblasts after co-cultivation with human lymphoid cells and 10% normal human serum. **D**, measles-infected fibroblasts after co-cultivation with human lymphoid cells and 10% SSPE serum. After termination the cultures were washed, fixed in ethanol and Giemsa stained. $\times 90$

The measles specific effect is found by subtracting the (per cent) cytotoxicity observed in the uninfected fibroblasts from that of the measles-infected fibroblasts.

Fig 1A shows a monolayer of measles-infected fibroblasts cultured for 72 hours in the absence of lymphocytes. Fig 1B shows corresponding fibroblast culture incubated with human lymphoid cells for 36 hours. Fig 1C shows uninfected control terminated after 36 hours' co-cultivation with the same lymphoid cell population.

It was found that in the presence of fetal calf serum or normal human serum, lymphocytes from SSPE patients were capable of killing measles-infected fibroblasts as effectively as lymphocytes from healthy control subjects. Similar observations have been reported by another group using a different cytotoxicity assay (Kreth *et al.* 1974). However, in the presence of an appropriate dilution of SSPE serum we have found that the cytotoxic effect of both SSPE and control lymphocytes can usually be substantially inhibited in our system (Fig 1D).

In some experiments interpretation has been difficult due to a high background killing of uninfected fibroblasts, while in others lymphocytes have had considerable feeder effect on the fibroblast cultures. We think, nevertheless, that we now have accumulated considerable evidence for the existence of a blocking factor in SSPE sera preventing the elimination of measles-infected cells. A rather similar conclusion has been reached by others using a different assay system (Sell *et al.* 1973). We have no direct evidence as yet that this blocking factor is antibody or antigen-antibody complex but this seems to be the most likely explanation. A possible mechanism of this blocking effect could be a loss of virus antigen expression on the fibroblast membranes. Thus, Dr M B A Oldstone and his colleagues have demonstrated that measles-infected cells, expressing virus antigen on their surface, will in the presence of measles antibody 'cap off' the virus antigens. These cells are then not able to regenerate the virus antigens on their surface as long as the antibody is present, but the

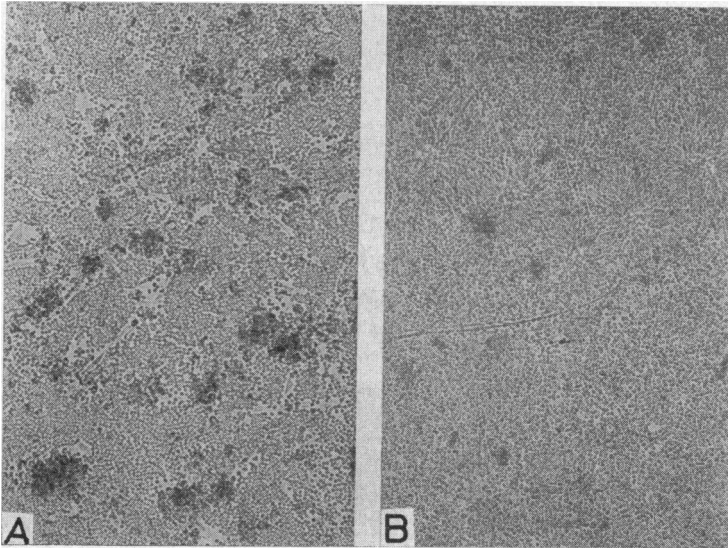


Fig 2 Measles-infected fibroblasts covered with lymphocytes. The photographs are taken through an inverted microscope after 24 hours co-cultivation: A, in the presence of normal human serum. B, in the presence of SSPE serum. $\times 75$

antigens will reappear after removal of the antibody from the culture medium (Joseph & Oldstone 1974).

We have observed that in the presence of normal serum lymphocytes seem to cluster around fibroblasts in the measles-infected cultures (Fig 2A). This clustering is minimal or absent when SSPE serum is present (Fig 2B).

We have also demonstrated that human lymphocytes will bind and form rosettes with measles-infected fibroblasts in suspension (Fig 3). The bound lymphocytes appear to be T cells since they are capable of binding sheep erythrocytes. If the lymphocyte population is depleted of T cells (less than 20% T cells) very few lymphocytes will bind in this system, and the few that do are not B lymphocytes, as judged by the absence of Fc receptors and surface immunoglobulins. Thus human T lymphocytes but not B lympho-

cytes seem to have a surface binding site for measles virus. On binding the T lymphocytes are cytotoxic to the measles infected cells regardless of whether the lymphocyte donor has had measles or not. Details of these findings will be published elsewhere (Valdimarsson, Agnarsson & Lachmann 1974, in preparation).

Transfer Factor Treatment

Since the principal immunological abnormality in SSPE is unduly high antibody titre associated with blocking of cellular immune response to measles, it seems rational to try to reduce the measles antibodies in the hope that this will lead to elimination of the infection. We are attempting to do this with transfer factor (TF), which might stimulate putative suppressor T cell function.

To date we have treated 8 SSPE patients with TF. While no definite clinical improvement has been observed so far, conversion of skin energy has been seen in most of these patients, and in 5 delayed skin reaction to measles antigen was achieved. In 3 patients conversion of *in vitro* reactivity with measles virus was also found as judged by lymphocyte transformation. However,

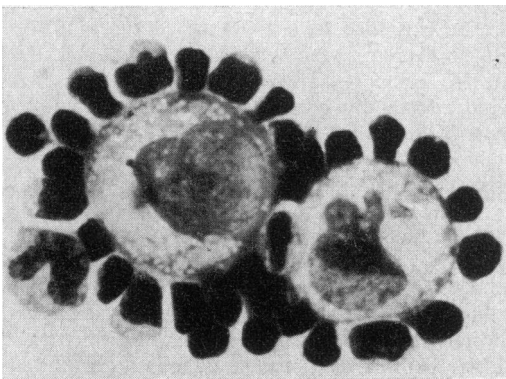


Fig 3 Measles rosettes consisting of measles-infected fibroblasts surrounded by human T lymphocytes

Table 1

Measles serum antibodies before and after transfer factor

Case	Before TF		After TF		No. of TF injections
	HI●	CF■	HI	CF	
JS	2048	2048	512	512	10
A K	1024	2048	256	512	5
P S	512	1024	256	1024	2
S W	1024	512	256	512	4
P F	512	1024	256	2048	2
M F	512	1024	256	512	2

● Hæmagglutinin inhibition antibodies
 ■ Complement fixing antibodies

the finding that impressed us most has been a fall in measles antibody titre in those patients who have been most intensively treated (Table 1). Therefore we feel that further trials of TF are warranted, especially for patients with early disease.

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DISCUSSION

Dr Valdimarsson in response to questioning said that the use of measles-infected cells rather than measles virus as an antigen in lymphocyte transformation tests had been reported by Sell and his colleagues (Ahmed *et al.*, 1974, *Journal of Experimental Medicine* **139**, 902) as giving better responses. However, the response to these measles-infected cells was maximal at three days which for antigen-induced response was surprising.

The question whether repeated skin testing alone would make delayed hypersensitivity responses positive was raised. Dr Valdimarsson said that he had not found this to be the case with the antigens that he had used. Skin testing could, however, induce lymphocyte transformation reactivity.

Dr Norrby on the question whether the virus in SSPE was truly measles or measles-like, quoted work from the Wistar Institute Group claiming differences between the SSPE virus and ordinary wild-type measles (ter Meulen *et al.* 1972, *Current Topics in Microbiology and Immunology* **57**, 1–38).

Dr Norrby, however, felt that the differences claimed might be due to a host modulation of normal measles virus and that many of these differences could be mimicked *in vitro* by passage in antibody-containing media. Furthermore, he pointed out that even the defective virus was capable of giving ferrets an SSPE-like disease.

On the question whether there was any association between H-LA phenotypes and SSPE, **Dr Platz** had HL-A typed 7 patients and **Dr Valdimarsson** 8 and no obvious association had shown up in either group.

Dr K Apostolov pointed out that cultures of brain explants from SSPE did not hæmadsorb monkey erythrocytes, indicating that these cells were not capable of assembling hæmagglutinin and budding off the virus (Payne *et al.*, 1969, *New England Journal of Medicine* **281**, 858). For this reason the virus could presumably spread only from cell to cell by cell fusion. Dr Apostolov said that the whole measles-

infected cell in this disease could be considered as a virus particle. He also raised the possibility that the process could be interrupted by giving complement intrathecally. There was some discussion of the mechanism of cytotoxicity of measles-coated cells.

Professor Lachmann quoted the work of M B Oldstone and his colleagues (personal communication) that measles-infected HeLa cells could be lysed by antibody and complement. The antibody could be either convalescent serum from normal measles or serum from patients with SSPE. Interestingly enough it was the alternative pathway of complement activation that appeared to be exclusively involved. If the cells were grown in an antibody containing medium in the absence of complement, the measles antigen 'capped' and was then extruded from the cell. Such cells, although still showing measles antigen inside them, no longer had antigen on the surface and could no longer be lysed by antibody and complement until after a period of growth in antibody-free medium.

Dr D K Peters pointed out that since the alternative pathway of complement activation was highly concentration-dependent there might not be enough complement in the CSF to produce lysis by this mechanism.

Dr Valdimarsson stated in his *in vitro* system there was some evidence to suggest that the background killing might be K cell killing, while the specific killing might be T cell killing.

Dr Erling Norrby

(Department of Virology, Karolinska Institutet, School of Medicine, Stockholm, Sweden)

and Dr Bodvar Vandvik

(Institute of Immunology and Rheumatology, and Department of Neurology, Rikshospitalet, Oslo, Norway)

Measles and Multiple Sclerosis¹

Adams & Imagawa (1962) were the first to propose a possible link between measles virus infections and multiple sclerosis (MS). This was based on the demonstration of relatively higher serum titres of antibodies against measles in MS patients than in matched controls. This difference has later been confirmed in a number of studies (cf. Brody *et al.* 1972). Further it was found that measles virus antibodies occurred in a much higher frequency in the cerebrospinal fluid (CSF) of MS patients than in healthy controls (Brown *et al.* 1971).

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