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# Specific and non-specific B cell activation in measles and varicella

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## SUMMARY

Lymphocytes from eight patients with measles and six patients with varicella were studied during the acute phase (first week) of illness and after recovery for spontaneous and pokeweed mitogen (PWM)-induced production of immunoglobulins (Ig) and viral antibodies by an enzyme linked immunosorbent assay (ELISA). In both infections acute phase lymphocytes showed increased spontaneous *in vitro* IgM and IgG productions including IgM and IgG antibodies to the aetiological virus as well as IgG antibodies to unrelated viruses (varicella, measles, rubella and mumps) to which the patient had serum antibodies. PWM induced no further Ig synthesis in the acute phase. In the convalescent phase viral antibody production could be demonstrated only in PWM stimulated cultures. In four patients the spontaneous synthesis of antibodies to a non-aetiological virus seemed to precede the production of IgG antibodies to the aetiological virus. All patients showed an increase of ELISA determined serum antibodies to the aetiological virus from the acute to the convalescent phase. Three of seven measles patients also showed a minor but significant increase or decrease of serum IgG antibodies to varicella and one of six varicella patients a significant rise of serum IgG antibodies to measles. Thus both measles and varicella infections were associated with non-specific as well as specific B cell activation. The non-specific B cell activation may be induced by non-specific helper factors from activated T cells.

## INTRODUCTION

It has been shown that a part of the immunoglobulins (Ig) appearing in response to experimental immunization or natural infection has no reactivity with the inducing antigen (Moticka & Streilein, 1978). Sensitive methods such as the enzyme linked immunosorbent assay (ELISA) can now be applied for the quantitation of *in vitro* Ig secretion and for determination of the specificity of the antibodies produced (Wasserman *et al.*, 1979; Biberfeld *et al.*, 1980). We have previously demonstrated production of viral antibodies in blood lymphocyte cultures of healthy donors stimulated *in vitro* by purified protein derivative of tuberculin (PPD) (Biberfeld *et al.*, 1980). In the present work we have studied spontaneous and pokeweed mitogen (PWM)-induced *in vitro* production of Ig and of viral antibodies in patients with measles or varicella infection. We here report that these two infections are associated with increased spontaneous Ig production and that the antibodies produced are directed not only at the aetiological virus but also at other, unrelated viruses with which the individuals have previously been infected.

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## MATERIALS AND METHODS

*Patients.* Heparinized blood was obtained from eight patients, 12–29 years old, in the acute phase of measles (1–5 days after the appearance of the rash) and from six patients, 23–36 years old, in the acute phase of varicella (3–6 days after the appearance of the rash). The mean age ± standard deviation (s.d.) in the measles and varicella patients as a group was 24·6 years ± 6·7. Six of the patients with measles were also tested after recovery (convalescent phase: more than 1 month after the onset of the symptoms) as were all the patients with varicella. Some of the patients were also examined 1 and 2 weeks after the first bleeding. All the patients had typical symptoms of measles or varicella without complications. The diagnoses were verified in all cases by the demonstration of a significant rise of IgG antibodies to the aetiological virus from the acute to the convalescent phase (see Results). As controls served 13 age matched and, with one exception, sex matched healthy persons, 19–36 years old (mean ± s.d.: 25·1 years ± 5·0), from the hospital staff. They were studied at the same occasions as the patients, each patient except one having his/her own control. One control was lost to follow up and two other controls were not studied a second time since the corresponding patients were not studied in the convalescent phase.

*Cell preparation.* Mononuclear cell suspensions were prepared by centrifugation of the blood on Ficoll-Isopaque (Ficoll-Paque, Pharmacia, Uppsala, Sweden; Böyum, 1976). The resulting suspension contained 5–10% polymorphonuclear cells and 3–30% monocytes (median value 13%) as identified by a non-specific esterase stain (Tucker, Pierre & Jordon, 1977). There was no significant difference in the relative proportion of monocytes between the acute and the convalescent phase. This suspension was used for the cell cultures.

*Culture conditions.* Cultures were set up in duplicate or triplicate in tubes containing $5 \times 10^5$ mononuclear cells in 1 ml of RPMI 1640 medium (GIBCO, Biocult, Paisley, Scotland) supplemented with 10% fetal calf serum (FCS; GIBCO Biocult), L-glutamine, penicillin and streptomycin. Some cultures were left unstimulated and some were stimulated with PWM; GIBCO, Grand Island, New York, USA) at a final dilution of 1/1,000 (1 μg/ml). The cultures were incubated at 37°C for 7 days in a humidified 5% $CO_2$ atmosphere. At the termination of culture the cells were centrifuged, the supernatants collected and stored frozen until tests for Ig and antibodies were performed.

*Ig determination by ELISA.* The amounts of IgG and IgM produced were measured in culture supernatants by a double antibody sandwich ELISA as previously described (Wasserman *et al.*, 1979).

*Determination of viral antibody activity by ELISA.* Viral antibodies were determined by an indirect antibody ELISA as previously described (Biberfeld *et al.*, 1980). The supernatants from the cell cultures were tested at a dilution of 1 : 5. A difference in absorbance of 0·15 between viral antigen and control antigen was significant. A change of absorbance of more than 0·30 between different sera from the same patient was regarded as significant.

*Viral antigens.* Measles, rubella and varicella antigens were prepared as previously described (Biberfeld *et al.*, 1980; Forghani, Schmidt & Dennis, 1978). Mumps antigen was prepared analogous to the measles antigen.

*Statistics.* The difference between acute phase and convalescent phase values were calculated and analysed by Wilcoxon's rank sum test. Only patients and controls tested in both the acute and convalescent phases were included in the statistical analysis (six patients with measles, six patients with varicella and 10 control persons).

## RESULTS

*Ig production* in vitro

The spontaneous *in vitro* IgG production was significantly increased during the acute phase (first week) of illness in both measles and varicella ($P < 0{\cdot}01$ for both diseases) (Table 1). The spontaneous IgM production was also increased but reached significance only in measles ($P < 0{\cdot}02$). The spontaneous Ig production decreased during the second week after the onset of the rash (only a few

**Table 1.** Spontaneous and PWM-induced IgM and IgG production in cultures of blood mononuclear cells from patients in the acute and convalescent phases of measles and varicella and from healthy controls.

| | | Ig concentration ng/ml × 100 | | | | Ratio | |
|---|---|---|---|---|---|---|---|
| | | IgM | | IgG | | PWM/medium | |
| | *n* | Medium | PWM | Medium | PWM | IgM | IgG |
| Measles | | | | | | | |
| Acute phase | 8 | 6·4 (0·6–25·0) | 6·5 (0·5–18·2) | 17·2 (3·7–44·0) | 9·5 (2·5–35·2) | 0·8 (0·7–1·1) | 0·7 (0·5–0·8) |
| Convalescent phase | 6 | 1·0 (0·3–1·3) | 7·6 (0·4–22·3) | 2·2 (1·3–3·4) | 6·9 (1·3–30·0) | 9·1 (1·0–20·0) | 2·4 (1·1–8·8) |
| Varicella | | | | | | | |
| Acute phase | 6 | 5·6 (0·1–14·4) | 4·7 (0·1–8·8) | 6·3 (1·9–130·0) | 7·9 (0·7–55·3) | 1·0 (0·5–2·1) | 0·5 (0·4–3·7) |
| Convalescent phase | 6 | 0·4 (0·1–2·9) | 4·8 (2·8–11·9) | 1·5 (0·7–2·8) | 4·8 (2·7–12·8) | 12·0 (4·0–59·0) | 4·5 (1·6–12·0) |
| Controls | | | | | | | |
| 1st test | 13 | 0·6 (0·2–14·0) | 6·3 (0·9–43·5) | 1·1 (0·5–11·4) | 4·8 (1·4–51·3) | 10·5 (1·7–30·0) | 4·1 (2·4–11·7) |
| 2nd test | 10 | 0·7 (0·1–4·7) | 2·3 (0·6–20·8) | 2·0 (0·4–6·4) | 5·1 (0·5–20·6) | 3·9 (1·5–41·6) | 2·1 (1·3–6·9) |

Medium = Ig production in cultures without PWM; PWM = PWM-induced Ig production; *n* = number of subjects investigated.
The values are median (range).

cases studied) (Table 3). The PWM-induced Ig production was not significantly different in the acute and the convalescent phase (Table 1). However, in the acute phase of both measles and varicella somewhat higher concentrations of IgM and IgG were usually found in unstimulated lymphocyte cultures than in PWM stimulated cultures (Table 1). Thus the ratios between the Ig production in PWM stimulated cultures and the Ig production in unstimulated cultures (PWM/medium) were significantly lower in the acute phase than in the convalescent phase (Table 1). (Measles: IgM; $P < 0{\cdot}02$, IgG; $P < 0{\cdot}01$. Varicella: IgM and IgG: $P < 0{\cdot}01$.)

*Viral antibodies in culture supernatants*

*Antibodies to the aetiological virus.* Spontaneously produced IgM and IgG antibodies to the aetiological virus were demonstrated in the acute phase (first week) of both diseases as shown in Table 2. In a few patients tested also during the second week after the onset of the rash, spontaneous viral IgM antibody production had ceased but spontaneous viral IgG antibody production persisted or had increased (Table 3). Seven of the eight patients with spontaneous production of IgG antibodies to the aetiological virus in acute phase cultures also had such antibodies in acute phase sera. The lowest serum dilution tested was 1/50. Four of the six patients who lacked spontaneous production of IgG antibodies to the aetiological virus in acute phase cultures also lacked such antibodies in acute phase sera. In PWM stimulated acute phase cultures, which usually had a somewhat lower Ig content than acute phase cultures without PWM (Table 1), antibodies to the aetiological virus were detected less frequently than in cultures without PWM (Table 2). In the convalescent phase viral antibodies were found only in PWM stimulated cultures (Table 2).

*Antibodies to non-aetiological viruses.* No spontaneous or PWM-induced production of IgM

**Table 2.** Antibodies to measles, varicella, rubella and mumps virus determined by ELISA in supernatants of unstimulated and PWM stimulated lymphocyte cultures from patients in the acute and convalescent phases of measles or varicella and from healthy controls

| | Number of subjects with detectable antibodies/number of subjects tested | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Unstimulated cultures | | | | | | PWM stimulated cultures | | | | | |
| | Antibodies to | | | | | | Antibodies to | | | | | |
| | Measles | | Varicella | | Rubella | Mumps | Measles | | Varicella | | Rubella | Mumps |
| | IgM | IgG | IgM | IgG | IgG | IgG | IgM | IgG | IgM | IgG | IgG | IgG |
| Measles | | | | | | | | | | | | |
| Acute phase | 3/3 | 4/8 | 0/2 | 6/8 | 2/3 | 1/3 | 3/3 | 2/8 | 0/2 | 6/8 | 2/3 | 1/3 |
| Convalescence | 0/2 | 0/6 | 0/2 | 0/6 | n.d. | n.d. | 0/2 | 5/6 | 1*/2 | 1/6 | n.d. | n.d. |
| Varicella | | | | | | | | | | | | |
| Acute phase | 0/3 | 4/6 | 2/3 | 4/6 | 1/3 | 0/3 | 0/3 | 4/6 | 2/3 | 2/6 | 1/3 | 0/3 |
| Convalescence | 0/3 | 0/5 | 0/3 | 0/5 | n.d. | n.d. | 0/3 | 3/5 | 0/3 | 5/5 | n.d. | n.d. |
| Controls | | | | | | | | | | | | |
| 1st test | 0/5 | 0/13 | 0/5 | 1†/13 | 0/2 | 0/2 | 0/5 | 5/13 | 0/5 | 8/13 | 0/2 | 0/2 |
| 2nd test | n.d. | 0/9 | n.d. | 0/9 | n.d. | n.d. | n.d. | 2/9 | n.d. | 4/9 | n.d. | n.d. |

* This patient had varicella 3 weeks after the measles.
† In one of duplicate culture tubes.
n.d. = not done.
All culture supernatants did not suffice for tests against all antigens.

**Table 3.** Total IgM and IgG and antibodies to measles and varicella determined by ELISA in supernatants of unstimulated (Med) and PWM stimulated (PWM) lymphocyte cultures from two patients with measles and one patient with varicella. (The viral IgG antibody content in the sera of patients 1 and 3 is shown in Table 4.)

| | | Total Ig ng/ml × 100 | | | | Antibody content expressed as absorbance | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Med | | | | PWM | | | |
| | | IgM | | IgG | | Measles | | Varicella | | Measles | | Varicella | |
| | Time after onset of rash | Med* | PWM | Med | PWM | IgM | IgG | IgM | IgG | IgM | IgG | IgM | IgG |
| Patient 1 | 2 days | 9·9 | 7·8 | 21·2 | 14·1 | 0·38 | <0·01 | <0·01 | >2·0 | 0·32 | <0·01 | <0·01 | >1·92 |
| (measles) | 11 days | 0·8 | 6·0 | 11·4 | 13·3 | <0·01 | 0·65 | <0·01 | <0·01 | 0·13 | 0·58 | <0·01 | 0·22 |
| Patient 3 | 5 days | 5·9 | 5·6 | 29·2 | 18·5 | n.d. | 0·60 | n.d. | 0·39 | n.d. | 0·40 | n.d. | 0·20 |
| (measles) | 11 days | 3·1 | 8·2 | 26·9 | 24·0 | n.d. | 0·42 | n.d. | 0·52 | n.d. | 0·42 | n.d. | 0·43 |
| | 2 months | 0·3 | 6·0 | 3·1 | 6·6 | n.d. | <0·01 | n.d. | <0·01 | n.d. | 0·47 | n.d. | <0·01 |
| Patient 6 | 6 days | 7·4 | 6·9 | 20·8 | 10·4 | <0·01 | 0·80 | 0·68 | 0·65 | <0·01 | 0·48 | 0·64 | 0·42 |
| (varicella) | 12 days | 0·2 | 6·0 | 2·5 | 5·2 | <0·01 | 0·10 | 0·02 | 0·78 | <0·01 | 0·10 | 0·31 | 0·46 |
| | 2 months | 2·9 | 11·9 | 2·8 | 12·8 | <0·01 | 0·04 | <0·01 | 0·04 | <0·01 | 0·13 | 0·08 | 0·72 |

The values are the mean of duplicate or triplicate cultures.
* Med (medium).

antibodies directed at a non-aetiological virus could be detected at any time (Table 2). Six of eight measles patients had IgG antibodies to varicella virus and four of six varicella patients had IgG antibodies to measles virus in unstimulated acute phase cultures (Table 2). In addition IgG antibodies to rubella or mumps virus were demonstrated in unstimulated acute phase cultures in a few patients studied (Table 2). Antibodies to a non-aetiological virus were found only in lymphocyte cultures of patients who had serum antibodies to the corresponding virus. In six cases serum antibodies to one of the non-aetiological viruses were lacking. Spontaneous *in vitro* production of IgG antibodies to one or more non-aetiological viruses was demonstrable in the acute phase cultures from three patients in the absence of IgG antibody production to the aetiological virus (see Table 2 and for example patient 1 in Table 3). In two of three patients tested again 1 week after the first test, the spontaneous production of IgG antibodies to a non-aetiological virus showed a decrease (Table 3) whereas the production of IgG antibodies to the aetiological virus showed an increase (patients 1 and 3 in Table 3). In the convalescent phase IgG antibodies directed at a non-aetiological virus were found only in PWM stimulated cultures (Table 2).

In controls no spontaneous or PWM-induced production of viral IgM antibodies was demonstrated (Table 2). IgG antibodies to a viral antigen were found in only one of the unstimulated lymphocyte cultures from healthy controls (Table 2). PWM-induced IgG antibodies to viral antigens were demonstrated in several of the control subjects (Table 2).

*Viral antibodies in serum*

All the patients had in the acute phase serum IgM antibodies to the virus causing their disease. Such antibodies were still detectable in the convalescent phase in most patients. In one measles patient varicella IgM activity in very low concentration was found 11 days after the onset of the rash. No other patient had serum IgM antibodies to a non-aetiological virus. All the patients developed a significant rise of IgG antibodies to the aetiological virus from the acute to the convalescent phase. One of six varicella patients had a significant rise of measles IgG antibodies from the acute to the convalescent phase (Table 4). Three of seven measles patients showed a significant increase or decrease of varicella antibodies (Table 4). In addition one measles patient who contracted varicella 3 weeks after the onset of measles had a significant increase of varicella antibodies. No significant

**Table 4.** Viral IgG antibody content in serum determined by ELISA and expressed as absorbance at the indicated serum dilution in the acute and convalescent phases in three patients with measles and in one patient with varicella

| | | Measles antibodies | | Varicella antibodies | |
|---|---|---|---|---|---|
| Patient number | Time after onset of rash | Serum dilution | Absorbance | Serum dilution | Absorbance |
| 1 | 2 days | 1:50 | 0·14 | 1:50,000 | 0·16 |
| (Measles) | 11 days | 1:5,000 | 0·75 | 1:50,000 | 0·69 |
| | 2 months | 1:5,000 | 1·53 | 1:50,000 | 0·28 |
| 2 | 3 days | 1:5,000 | 0·18 | 1:5,000 | 0·88 |
| (Measles) | 5 months | 1:5,000 | >2·0 | 1:5,000 | 0·38 |
| 3 | 5 days | 1:5,000 | 0·51 | 1:5,000 | 0·70 |
| (Measles) | 2 months | 1:5,000 | 1·04 | 1:5,000 | 1·02 |
| 4 | 3 days | 1:50,000 | 0·29 | 1:500 | 0·69 |
| (Varicella) | 1 month | 1:50,000 | 0·65 | 1:5,000 | 1·82 |

A change of absorbance of more than 0·30 representing two-fold titre difference, was regarded as significant.

changes of the serum antibody levels were found in the controls. In the controls the median difference between the absorbance values in the first and in the second serum was for measles antibodies 0·06 (range 0·02–0·12) and for varicella antibodies 0·11 (range 0·01–0·26).

## DISCUSSION

In the present study we have demonstrated increased spontaneous Ig production in lymphocyte cultures from patients in the acute phase of measles or varicella. This Ig response was both specific and non-specific as shown by the demonstration not only of antibodies to the aetiological virus but also of antibodies to unrelated viruses. The non-specific B cell response in these infections may be the result of viral-induced T cell activation with release of non-specific B cell activating factors. T cell derived non-antigen specific helper factors of B cell differentiation were first described in the mouse (Schimpl & Wecker, 1972). In man, production of B cell activating factors has also been demonstrated after *in vitro* antigenic stimulation, for example with PPD or tetanus toxoid, of blood T cell from healthy donors previously immunized with the specific antigen (Blomgren, 1975; Geha, 1979). These helper factors have been shown to stimulate B cells both to DNA synthesis (Blomgren, 1975; Geha, 1979) and Ig production, part of which does not have specificity for the inducing antigen (Geha, 1979). We have previously demonstrated T cell-dependent viral antibody production in PPD stimulated lymphocyte cultures from tuberculin skin test positive healthy donors (Biberfeld *et al.*, 1980). Similar results were obtained using tetanus toxoid stimulated lymphocyte cultures from tetanus toxoid immunized donors (Arneborn & Biberfeld, unpublished data). During measles an increase of the spontaneous production of B cell activating factor(s) (lymphocyte mitogenic factor) has been found (Joffe & Rabson, 1981).

There is no evidence that measles or varicella virus acts as a T cell-independent polyclonal B cell activator as has been shown for Epstein-Barr virus (Bird & Britton, 1979).

The production of antibodies to non-aetiological viruses in viral infections could be due to subclinical activation of latent viruses as a result of viral-induced immunosuppression. Varicella zoster virus is well known to cause latent infections. However, latent measles, rubella or mumps virus infections are, to our knowledge, infrequent and still we found spontaneous *in vitro* production of antibodies to one or more of these viruses in most patients with varicella and production of rubella or mumps antibodies in addition to varicella antibodies in patients with

measles. This makes activation of latent infections unlikely as a general explanation of the phenomenon.

We found that the antibodies to non-aetiological viruses spontaneously produced in cultures from measles or varicella patients were of IgG class and directed only at viruses to which the donor was seropositive, thus reflecting immunological memory. We also observed that in some cases the spontaneous *in vitro* production of antibodies to non-aetiological viruses seemed to precede the production of antibodies to the aetiological virus. These findings are in general agreement with studies of non-specific Ig responses in immunized animals (Moticka & Streilein, 1978).

An increased spontaneous production of antibodies to one or more non-aetiological viruses was demonstrated in lymphocyte cultures from 10 of 14 patients with measles or varicella but a significant change in the serum level of such antibodies from the acute to the convalescent phase was detected in only a few of these patients. Reasons for this might be that the 'anamnestic' response to non-aetiological viruses developed early in infection and that the sera were not tested at an optimal time for the detection of increases of this response. Furthermore, it may be difficult to detect an increase of antibodies if the preexisting serum level of these antibodies is fairly high.

In a recent study of patients with mumps, meningitis intrathecal antibody responses to viral antigens other than mumps virus were demonstrable (Vandvik *et al.*, 1982); observations that are in line with the demonstration of non-specific B cell responses in the viral infections of the present study.

Previously we have shown that vaccine-induced rubella infection causes a suppression of the PWM-induced Ig production in lymphocyte cultures (Arneborn, Biberfeld & von Stedingk, 1982). In the present patients with measles or varicella no decrease of the Ig production in PWM stimulated acute phase lymphocyte cultures was observed as compared to the Ig production in PWM stimulated convalescent phase cultures. However, in the acute phase the Ig production in PWM stimulated cultures was not higher but rather somewhat lower than the spontaneous Ig production in unstimulated cultures. One reason for this lack of PWM responsiveness could be that most B cells had been activated to plasmacytoid maturation *in vivo* as indicated by the high spontaneous Ig production. Immunofluorescence studies of smeared uncultured blood mononuclear cells from the acute phase of measles (four cases) and varicella (one case) showed that 8–15% of the cells were positive for intracytoplasmic Ig as compared to 0·1–0·4% of the blood mononuclear cells in three control subjects (Arneborn & Biberfeld, unpublished data). A defective PWM-induced production of helper factors for B cell proliferation, as has been demonstrated in patients with measles (Joffe & Rabson, 1981), may have contributed to the lack of a PWM response. It is possible that there was also an active suppression of the PWM response induced by suppressor cells. In a parallel study we have determined T lymphocyte subpopulations in the present patients and shown that varicella but not measles was associated with an increase of T cells of the suppressor/cytotoxic cell phenotype in the acute phase of illness (Arneborn & Biberfeld, 1983).

In summary we have shown that measles and varicella infections stimulate the production of antibodies directed not only at the aetiological virus but also at other unrelated viruses. This may be a general way of reacting to antigenic stimulation which may help in maintaining immunity to previously experienced infections (Moticka & Streilein, 1978).

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