Non-Specific Polyclonal Antibody Response Induced by  
*Mycoplasma pneumoniae*

GUNNEL BIBERFELD, M.D., Ph.D., a PER ARNEBORN, M.D., Ph.D., b  
MARIANNE FORSGREN, M.D., Ph.D., b  
LARS-VIKTOR VON STEDINGK, Ph.D., c AND SVEN BLOMQVIST, M.D. b

aDepartment of Immunology, National Bacteriological Laboratory, Stockholm;  
bDepartment of Infectious Diseases, Karolinska Institutet, Roslagstull Hospital, Stockholm;  
cCentral Microbiological Laboratory of Stockholm County Council, Stockholm, Sweden

Received January 4, 1983

The ability of heat-killed *Mycoplasma pneumoniae* (MP) organisms to induce polyclonal antibody production in cultures of blood lymphocytes of healthy subjects was studied. MP induced both IgM and IgG production, with a predominance of IgM. Supernatants of MP-stimulated lymphocyte cultures were tested by an enzyme-linked immunosorbent assay for antibodies to measles, rubella, and herpes simplex virus. MP as well as pokeweed mitogen induced production of viral antibodies of IgG class in lymphocytes of donors who had serum antibodies to the corresponding viral antigens. The MP-induced non-specific antibody response was T-cell-dependent. Lymphocytes from four patients with MP pneumonia, collected nine to 13 days after onset of illness, were tested for *in vitro* Ig production in the absence of MP. These lymphocytes spontaneously produced increased amounts of IgM and/or IgG. Lymphocytes from three of these four patients spontaneously produced viral IgG antibodies to measles and/or varicella antigens, indicating that MP had induced non-specific activation of memory B cells *in vivo*. Spontaneous viral antibody production was not found in lymphocyte cultures of healthy donors. The non-specific activation of blood B cells *in vitro* is probably induced by non-specific helper factors from MP-activated T cells. It is possible that *in vivo* MP also may have a direct activating effect on B cells.

INTRODUCTION

*Mycoplasma pneumoniae* (MP) infection in man is associated with an increase of serum immunoglobulins that is only in part due to specific MP antibodies [1]. MP has been shown to be a polyclonal non-specific B-cell activator in the mouse [2].

In the present work we have studied the polyclonal antibody response *in vitro* of human lymphocytes activated by MP *in vitro* or *in vivo* using the sensitive enzyme-linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

Heparinized blood was collected from healthy donors and from patients with MP pneumonia. Lymphocytes were purified and cultured as described previously [3]. The procedures used are here described only briefly. Mononuclear cells were separated from blood by Ficoll-Isopaque gradient centrifugation. In several ex-
Experiments the cells were further purified by removal (incomplete) of adherent cells [3], leaving less than 4 percent of monocytes in the suspension. B- and T-cell-enriched fractions were prepared by rosetting of lymphocytes with neuraminidase-treated sheep red blood cells, followed by gradient centrifugation [3]. Blood mononuclear cells or lymphocytes with a low content of adherent cells were cultured at a concentration of 5 × 10⁴ cells per ml and tube in the presence or absence of antigen or mitogen in RPMI-1640 medium supplemented with 10 percent fetal calf serum for seven days at 37°C in humidified air with 5 percent CO₂. The following antigens or mitogens were used in lymphocyte cultures: (1) a sonicated concentrate of MP organisms [4], heat-killed for 30 minutes at 56°C, at a final concentration of 1 to 10 μg protein/ml; (2) purified protein derivative of tuberculin (PPD) at a final concentration of 100 μg/ml (most people in Sweden are sensitized to PPD tuberculin since it was common practice until 1975 to vaccinate newborns with Bacillus-Calmette-Guerin); (3) pokeweed mitogen (PWM) at a final concentration of 1 μg/ml.

Culture supernatants were collected after seven days of culture and tested for total IgM and IgG and for antibodies to viral antigens (measles, varicella, rubella, mumps, and herpes simplex) by ELISA [3,5]. The supernatants were diluted 1:5 for viral antibody tests.

RESULTS

In blood lymphocyte cultures of healthy donors MP as well as PWM and PPD induced IgM and IgG production (Table 1). The MP-induced IgM response was of the same magnitude as that induced by PWM, whereas the IgG response was lower than

| Stimulator | IgM (ng/ml) | IgG (ng/ml) |
|------------|-------------|-------------|
| MP         | 4,946 (2,600-8,500) | 2,438 (380-5,300) |
| PWM        | 5,416 (1,328-6,336) | 5,210 (1,230-9,880) |
| PPD        | 9,254 (2,624-17,200) | 2,853 (1,470-4,020) |
| None       | 804 (160-1,440) | 383 (120-640) |

Viral IgG Antibody Production in Eight Healthy Donors

| Stimulator | Measles | Rubella | Herpes | Any of the antigens |
|------------|---------|---------|--------|---------------------|
| MP         | 4/8     | 4/8     | 0/8    | 6/8                 |
| PWM        | 5/8     | 7/8     | 5/8    | 8/8                 |
| PPD        | 4/8     | 7/8     | 3/8    | 8/8                 |
| None       | 0/8     | 0/8     | 0/8    | 0/8                 |

*The cells had been partially depleted of adherent cells.

*All healthy donors had in serum ELISA antibodies to measles and rubella virus (titers ≥ 5,000) and five of eight donors had antibodies to herpes simplex virus (titer ≥ 5,000).
NON-SPECIFIC ANTIBODIES AND M. PNEUMONIAE

TABLE 2
Total IgG and Viral IgG Antibodies in Supernatants of in Vitro Stimulated Lymphocyte Cultures of a Healthy Donor*

| Stimulator | IgG ng/ml | Measles | Rubella | Herpes simplex |
|------------|-----------|---------|---------|---------------|
| MP         | 3,460     | 1.25    | 0.89    | <0.15         |
| PWM        | 5,200     | 1.15    | 1.29    | <0.15         |
| PPD        | 4,020     | 0.70    | 0.51    | <0.15         |
| None       | 460       | <0.15   | <0.15   | <0.15         |

*The ELISA antibody titer in serum was >50,000 against measles and rubella and <50 against herpes simplex virus.

that stimulated by PWM. MP as well as PWM and PPD induced in vitro production of IgG antibodies to various viral antigens to which the lymphocyte donors had serum antibodies (Tables 1 and 2). The MP-induced non-specific antibody response was T-cell-dependent (Table 3).

Lymphocytes from four patients (28 to 46 years old) with MP pneumonia were also tested for in vitro Ig production. Lymphocytes collected nine to 13 days after onset of illness spontaneously produced increased amounts of IgM and IgG as exemplified in Table 4. In three of these four patients, ELISA IgG antibodies to

**TABLE 3**
T-Cell Dependence of MP- and PPD-Induced Ig and Viral Antibody Production in Vitro (Lymphocytes from a Healthy Donor)

| Stimulator | IgG ng/ml | B cells only | Antibody to rubella | B + T cells | IgG ng/ml | Antibody to rubella |
|------------|-----------|--------------|---------------------|-------------|-----------|---------------------|
| MP         | 90        | 0.02*        | 2,280               | 0.62*       |
| PPD        | 90        | 0.02*        | 3,280               | 1.11*       |
| None       | 60        | 0.02*        | 280                 | 0.01*       |

*Absorbance

**TABLE 4**
Spontaneous Ig and Viral Antibody Production in Lymphocyte Cultures* of a Patient with MP Pneumonia Studied 13 Days after Onset of Illness

|          | IgM ng/ml | IgG ng/ml | ELISA IgG antibodies (absorbance) |
|----------|-----------|-----------|-----------------------------------|
| MP patient* | 4,540     | 2,200     | Measles 0.78 Varicella 0.32       |
| Median (range) in 10 healthy donors | 70 (10–470) | 200 (40–640) | <0.15 <0.15 |

*The lymphocytes were cultured for seven days.

*The patient as well as the healthy donors had serum antibodies to measles and varicella.
measles and/or varicella antigens were demonstrated in unstimulated lymphocyte cultures (i.e., cultures without MP, PWM, or PPD). One example is shown in Table 4. All patients had serum antibodies to measles and varicella as evidence of past infection with these viruses. Spontaneous in vitro viral antibody production was not found in lymphocyte cultures of healthy donors (Table 4).

The Ig content in unstimulated lymphocyte cultures was higher in the healthy donors shown in Table 1, where adherent cell-depleted cultures had been used, than in the healthy donors shown in Table 4. In our hands, unstimulated as well as antigen-stimulated lymphocyte cultures partially depleted of adherent cells often have a higher Ig content than cultures not depleted of adherent cells.

**DISCUSSION**

The MP-induced non-specific activation of human blood B cells in vitro was found to be T-cell-dependent and is probably induced by non-specific helper factors from MP-activated T cells. Production of non-specific B-cell activating factors has been demonstrated after in vitro stimulation with tetanus toxoid (TT) of blood T cells from healthy donors previously immunized with TT [6]. The non-specific anamnestic B-cell response induced by MP in vivo may also be caused by non-specific T helper factors. It is possible that MP in addition has a direct activating effect on B cells in vivo. We have previously demonstrated activation of anamnestic IgG antibody responses to non-etiological viruses in patients with measles or varicella infections [5]. Non-specific activation of memory B cells may be a common phenomenon following a strong antigenic stimulation of T cells, which may help in maintaining memory to previously experienced infections [7]. Such non-specific anamnestic B-cell responses may occasionally cause difficulties in diagnosing infections by serology.

**ACKNOWLEDGEMENTS**

This work was supported by the Swedish Medical Research Council (project 16X-02380), the Swedish Society of Medical Sciences, and Karolinska Institutet.

**REFERENCES**

1. Biberfeld G: Antibody responses in Mycoplasma pneumoniae infection in relation to serum immunoglobulins, especially IgM. Acta Path Microbiol Scandinav B 79:620–634, 1971
2. Biberfeld G, Gronowicz E: Mycoplasma pneumoniae is a polyclonal B-cell activator. Nature 261:238–239, 1976
3. Biberfeld G, Forsgren M, von Stedingk L-V, et al: PPD-induced viral antibody production in human blood lymphocytes. Clin Exp Immunol 42:364–369, 1980
4. Biberfeld G: Activation of human lymphocyte subpopulations by Mycoplasma pneumoniae. Scand J Immunol 6:1145–1150, 1977
5. Arneborn P, Biberfeld G, Forsgren M, et al: Specific and non-specific B cell activation in measles and varicella. Clin Exp Immunol 51:165–172, 1983
6. Geha RS: Regulation of human B cell activation. Immunol Rev 45:275–305, 1979
7. Moticka EJ, Streilein JW: Hypothesis: nonspecific polyclonal activation of memory B cells by antigen as a mechanism for the preservation of long term immunologic anamnesis. Cell Immunol 41:406–413, 1978