Decreased Oxygen Radical Generation by Neutrophils from Patients with Measles Presumably Owing toActivation of Suppressor T Lymphocytes

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The capacity for the generation of oxygen radicals by polymorphonuclear leukocytes (PMNs) was assessed in 29 patients with measles and in control groups. Patients with secondary bacterial infections showed a significantly decreased generation of oxygen radicals; this abnormality did not persist for more than 2 months after disease onset. Normal PMNs incubated with T lymphocytes from these measles patients generated significantly fewer oxygen radicals than those incubated with T cells from either control group. However, normal PMNs incubated with non-T lymphocytes from these measles patients produced normal oxygen radical levels. In addition, irradiation above 1,500 rads of T lymphocytes abrogated the suppressive effect of T cells on PMNs. On the other hand, these abnormal findings were not observed in patients with measles but without secondary bacterial infections. The secondary bacterial infections sometimes seen in measles patients may result from a decrease in oxygen radical generation, presumably induced by the suppressive activity of the T lymphocytes of the patients.

The decreased generation of oxygen radicals by polymorphonuclear leukocytes (PMNs) has been reported in some viral diseases (14). It has recently been documented (5a) that T lymphocytes from patients with infectious mononucleosis suppress oxygen radical generation by PMNs.

In some but not all patients with measles, we observed a significant decrease in oxygen radical generation. We investigated the capacity for the generation of oxygen radicals by their PMNs and the suppressive activity of their T lymphocytes on oxygen radical generation by PMNs.

MATERIALS AND METHODS

Subjects. The study population consisted of 29 patients with measles (15 males, 4 to 17 years old, and 14 females, 4 to 19 years old). All were previously healthy. The reason that the patients were over 4 years old is the limited amount of blood that can ethically be drawn from any one subject. Six of the patients were affected with secondary bacterial infections, such as bacterial pneumonia, urinary tract infections, and otitis media. These six patients were treated as a different group. The secondary bacterial infections were diagnosed by a clinical examination and by the isolation and identification of pathogenic organisms from appropriate body sites by culturing sputum, arterial blood, urine, or pus discharge samples from the patients. Staphylococcus aureus and Haemophilus influenza type were identified as being responsible for the pneumonia in four patients, and a pneumococcus was responsible for a case of otitis media. These patients were treated with antibiotics such as sodium cephalosporins, sulbenicillin, and cephalaxin. No previous bacterial infections had been noted in these six patients for at least 6 months before the investigation. All secondary bacterial infections subsided within 6 weeks.

The controls were 10 sex- and age-matched healthy volunteers and 15 patients with influenza, varicella (chicken pox), mumps, or herpes zoster (cutaneous). The exact diagnosis of measles and influenza was made by the hemagglutination inhibition test, and that of varicella, mumps, and herpes zoster was made by the complement fixation test. The control patients with viral infections and the patients with measles had serious general symptoms, such as a high fever (above 37°C) and/or a generalized skin rash or vesicle formation. None of the subjects had taken aspirin, indomethacin, or glucocorticosteroids 48 h before the assays were done. The initial assays for oxygen radical generation in measles patients were performed on the day on which Koplik's spots clinically appeared and were repeated at biweekly intervals until the values returned to normal. The control patients with viral infections were assessed for oxygen radical generation within 3 days after the onset of symptoms.

Oxygen radical generation assays. PMNs were isolated from peripheral blood by Ficoll-Hypaque gradient centrifugation as previously described (4, 7; Y. Niwa, T. Sakane, M. Shingu, I. Yanagida, J. Komura, and Y. Miyachi, Arch. Dermatol., in press). The methods used for assaying oxygen radical generation have been detailed in previous reports (4, 6, 7; Niwa et al., Arch. Dermatol., in press). Briefly, the formation of O₂⁻ was determined by measuring ferricytochrome c reduction induced by O₂⁻ produced from PMNs stimulated with 1 mg of opsonized zymosan per ml; the absorbance was measured at 550 nm. The generation of H₂O₂ was determined with opsonized-zymosan-stimulated PMNs, scopoletin, and horseradish peroxidase; the rate of decrease in the fluorescence intensity of the scopoletin within 30 min was quantitated in a fluorescence spectrophotometer. Hydroxyl radicals (OH⁻) were quantitated by measuring the amount of ethylene gas formed from α-ketomethiol butylic acid and by measuring the amount of PMN-generated OH⁻: the total amount of OH⁻ gas formed at 10, 20, and 30 min was determined on a gas chromatograph.

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Vol. 21, No. 3
JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1985, p. 318–322
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Chemiluminescence was measured in a scintillation spectrometer with opsonized-zymosan-stimulated PMNs but no luminol in the dark. In each oxygen generation assay system, PMNs not stimulated by opsonized zymosan were simultaneously tested.

**Incubation of PMNs with T lymphocytes.** Adherent cells (monocytes) and nonadherent cells (lymphocytes) were further separated, by incubation on petri dishes (9), from the mononuclear cell fractions which were obtained simultaneously with PMNs. Adherent-cell preparations were identified as 95% monocytes by Giemsa staining and by reaction with OKM-1 monoclonal antibody (Ortho Pharmaceutical Corp., Raritan, N.J.), which binds to cells of the monocyte and myeloid series. The T-lymphocyte fraction was isolated by the sheep erythrocyte rosetting technique (2) and contained less than 1% monocytes. The nonsetting population, consisting mostly of B lymphocytes and null cells, was referred to as the non-T-cell population; it contained 13 ± 3.5% monocytes. To examine the effect of T cells on the generation of oxygen radicals by PMNs, purified T lymphocytes (from the measles patients or controls) supplemented with 5% mitomycin-treated monocytes were cocultured with PMNs (from healthy individuals) at 37°C for 17 h. Previous studies (6, 7) and preliminary experiments in the present study showed that 17 h is the optimal incubation time for inducing changes in oxygen radical generation in PMN-T-lymphocyte cocultures without inducing a decrease in the viability and phagocytic functions of PMNs. Before and after coculture with T lymphocytes, the viability of PMNs was assessed, and related data were discarded when PMNs showed less than 98% viability in the trypsin blue exclusion test or fewer than 600 dpm/mg of [14C]inulin uptake as a measurement of phagocytic activity (6, 12, 13).

The effect of oxygen radicals generated by a small number of added or contaminating monocytes in T-cell-PMN cocultures was negligible, because monocytes generate far fewer oxygen radicals than PMNs (9). The coculture ratio of PMNs to T lymphocytes (6, 7) was 3:1 in RPMI 1640 medium containing 20% heat-inactivated pooled human AB serum. PMNs isolated from T lymphocytes by the Ficoll-Hypaque gradient method or PMNs in the presence of T lymphocytes were examined for oxygen radical generation as already described. Control incubations (17 h, 37°C) of T lymphocytes from healthy individuals with autologous or allogeneic normal PMNs were performed in a similar way. Another control consisted of the addition of non-T mononuclear cells from measles patients to PMNs from healthy individuals. Further, because suppressor T cells are sensitive to irradiation (11), we examined whether irradiation (1,000, 1,500, 2,000, or 2,500 rads) of the T-cell populations from the patients could abrogate the suppressive effect of the T cells on oxygen radical generation by normal PMNs.

**Statistical analysis.** Triplicate assays were performed simultaneously for each experiment, and the results were expressed as the mean ± standard error of the mean. The statistical significance was ascertained by Student's t test.

**RESULTS**

**Oxygen radical generation by PMNs.** PMNs from all measles patients showed a significantly decreased generation of oxygen radicals as compared with PMNs from healthy control groups (Table 1). Measles patients who were affected with secondary bacterial infections showed much less oxygen radical generation by PMNs than did both virus-infected and healthy controls. They also showed less generation than did measles patients who had no secondary bacterial infections. However, PMNs from measles patients without secondary bacterial infections did not generate significantly lower oxygen radical levels than did PMNs from healthy controls (P > 0.05) and showed oxygen radical generation levels almost equal to those in patients with other viral infections. Oxygen radical generation by PMNs from patients with other viral infections was slightly decreased, but not significantly when compared with levels in healthy controls (P > 0.05). When oxygen radical generation by nonstimulated PMNs was assayed, findings similar to those with stimulated PMNs were obtained (Table 1).

Decreased oxygen radical generation by PMNs from measles patients with secondary bacterial infections returned to normal values within 8 weeks of the onset of disease (Fig. 1).

**Coincubation of normal PMNs with lymphocytes from patients.** PMNs (from healthy individuals) incubated with T lymphocytes from six measles patients with secondary bacterial infections generated significantly decreased oxygen radical levels, as compared with PMNs incubated with T lymphocytes from allogeneic healthy individuals (Table 2). However, normal PMNs incubated with T lymphocytes from patients with measles but without secondary bacterial infections and from patients with viral infections showed no decrease in oxygen radical generation (P > 0.05), as compared with PMNs incubated with normal allogeneic T lymphocytes.

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**TABLE 1. Generation of oxygen radicals by PMNs from patients and control groups with or without (data in parentheses) stimulation by opsonized zymosan**

<table>
<thead>
<tr>
<th>Disease (no. of subjects tested)</th>
<th>O$_2^*$ (pmol $\times 10^1$/min per 4 x $10^8$ PMNs)</th>
<th>H$_2$O$_2$ (pmol $\times 10^2$/min per 2.5 x $10^8$ PMNs)</th>
<th>OH$^\cdot$ (pmol $\times 10^2$/5 x $10^8$ PMNs)</th>
<th>Chemiluminescence (cpm $\times 10^4$/5 x $10^8$ PMNs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measles (29)</td>
<td>4.2 ± 0.85$^a$</td>
<td>3.81 ± 0.48$^a$</td>
<td>6.43 ± 0.69$^a$</td>
<td>14.7 ± 2.8$^a$</td>
</tr>
<tr>
<td></td>
<td>(0.14 ± 0.03)$^a$</td>
<td>(0.86 ± 0.05)$^a$</td>
<td>(0.069 ± 0.004)$^b$</td>
<td>(1.78 ± 0.03)$^b$</td>
</tr>
<tr>
<td>Measles without secondary bacterial infections (23)</td>
<td>4.8 ± 0.61$^a$</td>
<td>4.10 ± 0.39$^a$</td>
<td>7.08 ± 0.32</td>
<td>16.4 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>(0.17 ± 0.05)$^a$</td>
<td>(0.91 ± 0.07)$^a$</td>
<td>(0.077 ± 0.005)</td>
<td>(1.94 ± 0.41)</td>
</tr>
<tr>
<td>Measles with secondary bacterial infections (6)</td>
<td>3.6 ± 0.31$^a$</td>
<td>3.02 ± 0.17$^a$</td>
<td>4.82 ± 0.25$^a$</td>
<td>10.7 ± 1.2$^a$</td>
</tr>
<tr>
<td></td>
<td>(0.12 ± 0.06)$^a$</td>
<td>(0.80 ± 0.06)$^a$</td>
<td>(0.041 ± 0.002)$^a$</td>
<td>(1.58 ± 0.12)$^a$</td>
</tr>
<tr>
<td>Other viral infections</td>
<td>4.9 ± 0.81</td>
<td>4.09 ± 0.41</td>
<td>7.15 ± 0.41</td>
<td>16.5 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>(0.16 ± 0.07)</td>
<td>(0.92 ± 0.06)</td>
<td>(0.080 ± 0.006)</td>
<td>(1.89 ± 0.2)</td>
</tr>
<tr>
<td>None (healthy controls)</td>
<td>5.6 ± 0.82</td>
<td>4.70 ± 0.30</td>
<td>7.75 ± 0.28</td>
<td>19.4 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>(0.20 ± 0.02)</td>
<td>(1.28 ± 0.09)</td>
<td>(0.097 ± 0.004)</td>
<td>(2.28 ± 0.13)</td>
</tr>
</tbody>
</table>

$^a$ 0.025 < $P$ < 0.05 versus healthy controls.

$^b$ 0.01 < $P$ < 0.025 versus healthy controls.

$^c$ $P$ < 0.01 versus healthy controls.

$^d$ $P$ < 0.001 versus healthy controls.
FIG. 1. Follow-up study of oxygen radical generation by PMNs from measles patients with secondary bacterial infections after the onset of disease. (a) Ferricytochrome c reduction (pmol × 10^6/min per 4 × 10^6 PMNs). (b) H_2O_2 generation (pmol × 10^6/min per 2.5 × 10^6 PMNs). (c) Ethylene formation (pmol × 10^2/2 × 10^6 PMNs). (d) Chemiluminescence (Chem.) (cpm × 10^5/5 × 10^6 PMNs).

phocytes (Table 2). PMNs separated from T lymphocytes after coincubation generated approximately the same levels of oxygen radicals as did PMNs not separated from T lymphocytes (Table 2). Lymphocytes alone from each subject failed to generate measurable oxygen radicals (data not shown).

**Characteristics of suppressor T cells.** Non-T-cell populations from any measles patient induced no decrease in oxygen radical generation by normal PMNs (data not shown). Further, the irradiation of T cells from measles patients with doses exceeding 1,500 rads abrogated their capacity to decrease oxygen radical generation by PMNs (Table 3). This suggests that a decrease in oxygen radical generation induced by coincubation with T lymphocytes may be a result of inhibition by suppressor-T-cell activity in measles patients.

PMNs (from healthy individuals) incubated with T lymphocytes (from allogeneic healthy individuals) generated almost the same levels of oxygen radicals as did PMNs cocultured with autologous T lymphocytes (Table 2). This observation ruled out the possibility that the decrease in oxygen radical generation influenced by T lymphocytes was the result of HLA differences between the PMN and lymphocyte donors.

**TABLE 2.** Opsonized-zymosan-stimulated generation of oxygen radicals by PMNs from healthy individuals after 17 h of incubation with T lymphocytes from patients and control groups

<table>
<thead>
<tr>
<th>T lymphocytes from (no. of subjects tested):</th>
<th>O_2^- (pmol × 10^6/min per 4 × 10^6 PMNs)</th>
<th>H_2O_2 (pmol × 10^6/min per 2.5 × 10^6 PMNs)</th>
<th>OH^- (pmol × 10^2/2 × 10^6 PMNs)</th>
<th>Chemiluminescence (cpm × 10^5/5 × 10^6 PMNs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measles with secondary bacterial infections (6)</td>
<td>4.1 ± 0.51^a</td>
<td>3.57 ± 0.22^a</td>
<td>5.02 ± 0.45^b</td>
<td>12.5 ± 1.4^a</td>
</tr>
<tr>
<td>Measles without secondary bacterial infections (10)</td>
<td>4.6 ± 0.62</td>
<td>4.08 ± 0.35</td>
<td>6.77 ± 0.52</td>
<td>14.9 ± 2.2</td>
</tr>
<tr>
<td>Other viral infections (10)</td>
<td>4.7 ± 0.58</td>
<td>4.25 ± 0.39</td>
<td>6.80 ± 0.64</td>
<td>14.8 ± 1.4</td>
</tr>
<tr>
<td>Allogeneic healthy individuals (7)</td>
<td>5.2 ± 0.81</td>
<td>4.48 ± 0.48</td>
<td>7.49 ± 0.53</td>
<td>17.8 ± 1.4</td>
</tr>
<tr>
<td>Autologous individuals (7)</td>
<td>5.3 ± 0.62</td>
<td>4.53 ± 0.51</td>
<td>7.31 ± 0.42</td>
<td>17.2 ± 1.0</td>
</tr>
<tr>
<td>Nonseparated leukocytes (5)^c</td>
<td>4.81 ± 0.51</td>
<td>4.22 ± 0.53</td>
<td>7.20 ± 0.67</td>
<td>16.4 ± 2.04</td>
</tr>
</tbody>
</table>

^a 0.01 < P < 0.05 versus control (oxygen radical levels generated by normal PMNs after coincubation with T lymphocytes from allogeneic healthy individuals).
^b P < 0.01 versus control (oxygen radical levels generated by normal PMNs after coincubation with T lymphocytes from allogeneic healthy individuals).
^c Oxygen radical generation was assessed for PMNs not separated from mononuclear fractions. In this assay, the ratio of PMNs to T lymphocytes was almost 3:1.
TABLE 3. Dose-dependent effect of irradiation on the suppressive activity of T lymphocytes from measles patients on opsonized-zymosan-stimulated generation of oxygen radicals by normal PMNs

<table>
<thead>
<tr>
<th>Radiation dose (rads)</th>
<th>O$_2^\cdot$ (pmol × 10$^{-6}$/min per 4 × 10$^6$ PMNs)</th>
<th>H$_2$O$_2$ (pmol × 10$^{-6}$/min per 2.5 × 10$^6$ PMNs)</th>
<th>OH$^\cdot$ (pmol × 10$^{-6}$/2 × 10$^6$ PMNs)</th>
<th>Chemiluminescence (cpm × 10$^5$/10$^6$ PMNs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.10 ± 0.51</td>
<td>3.57 ± 0.22</td>
<td>5.02 ± 0.45</td>
<td>12.5 ± 1.4</td>
</tr>
<tr>
<td>1,000</td>
<td>4.30 ± 0.21</td>
<td>3.72 ± 0.30</td>
<td>5.53 ± 0.53</td>
<td>13.8 ± 1.9</td>
</tr>
<tr>
<td>1,500</td>
<td>4.70 ± 0.24</td>
<td>4.01 ± 0.27</td>
<td>6.42 ± 0.32</td>
<td>16.5 ± 0.8</td>
</tr>
<tr>
<td>2,000</td>
<td>4.90 ± 0.28</td>
<td>4.28 ± 0.30</td>
<td>6.71 ± 0.41</td>
<td>16.8 ± 1.8</td>
</tr>
<tr>
<td>2,500</td>
<td>5.10 ± 0.30</td>
<td>4.31 ± 0.32</td>
<td>7.22 ± 0.50</td>
<td>17.0 ± 2.2</td>
</tr>
</tbody>
</table>

* PMNs from six healthy individuals were coincubated at 37°C for 17 h with T lymphocytes from six measles patients with secondary bacterial infections. T lymphocytes were irradiated immediately before they were cocultured with PMNs.

* 0.01 < P < 0.05.

* P < 0.01 versus control (oxygen radical levels generated in PMN-T-lymphocyte cocultures without irradiation).

Although the increased ratio of T-lymphocytes from measles patients to normal PMNs in our coculture experiment induced a decrease in oxygen radical generation by normal PMNs, the increase in the T-lymphocyte/PMN ratio for any control group did not significantly affect oxygen radical generation by normal PMNs (data not shown). A decrease in oxygen radical generation by normal PMNs was observed in control groups only after incubation of PMNs with a markedly increased amount of T lymphocytes. Various changes in the ratios of non-T lymphocytes to PMNs also did not significantly change our results (data not shown).

**DISCUSSION**

The present study revealed that the capacity for oxygen radical generation by PMNs is decreased in patients with measles and secondary bacterial infections. In viral infections, such as infectious mononucleosis, it is known that T lymphocytes serve as an important control mechanism in suppressing Epstein-Barr virus-induced B-cell proliferation (1, 10, 15-17) as well as serving as a target of the causative virus. It has recently been suggested (5a) that T lymphocytes from patients with infectious mononucleosis inhibit PMN function in these patients. Although the activation of the suppressive activity of T lymphocytes from measles patients has not been clarified, the present study provides evidence for the suppressive effect of T lymphocytes from measles patients on the generation of oxygen radicals by their PMNs. We were unable to determine T-cell subsets in relation to suppressive activity on oxygen radical generation by PMNs from measles patients, as examined with PMNs from infectious mononucleosis patients. However, findings similar to those observed for infectious mononucleosis patients were observed in the present study for measles patients, including the abrogation of suppressive T-cell activity on oxygen radical generation by irradiation and no induction of a decrease in oxygen radical generation by the addition of non-T-cell populations from any measles patient. Thus, it is possible that mechanisms of action similar to those of OKT4$^+$ cells on oxygen radical generation by PMNs might be present in measles patients.

In addition to the enhanced suppressive activity of T lymphocytes in patients with infectious mononucleosis, several immune abnormalities have been reported in rubella (3) and common viral diseases (5). Immune disturbance has also been documented in measles (5). Both immunological abnormalities in viral diseases (3, 5), including measles, and a decrease in oxygen radical generation by PMNs in infectious mononucleosis patients demonstrated in a recent investigation (5a) abated within 2 months. This fact coincides with the results of our follow-up studies of oxygen radical generation by PMNs in measles patients. This time course supports the speculation that the reduced oxygen radical generation may result from the enhanced suppressive activity of T lymphocytes. Our present study also suggests the possibility that secondary bacterial infections in measles patients may be a result of a decrease in oxygen radical generation by PMNs. Additionally, it can be speculated that the combination of bacterial and rubella virus infections caused a greater T-lymphocyte-induced decrease in oxygen radical generation by PMNs than rubella virus infection alone. Solberg et al. (14) reported that oxygen radical generation by PMNs increased in patients with bacterial infections but decreased in patients with viral infections. Their findings for patients with bacterial infections were almost consistent with ours and with those of others (5a, 6, 8; Niwa et al., Arch. Dermatol., in press); however, their findings for patients with viral infections were not. It has been demonstrated elsewhere (5a) and in this study that PMNs from patients with viral infections do not generate significantly decreased oxygen radical levels when infectious mononucleosis and measles are excluded from the study.

Further studies are required to verify the activation of suppressor T lymphocytes from measles patients against target cells other than PMNs and to elucidate the mechanism of suppressive T-cell activity on PMNs. The different immunological behavior of the disease with a decreased capacity for oxygen radical generation by PMNs (infectious mononucleosis and measles with secondary bacterial infections) and those without a decreased capacity for oxygen radical generation by PMNs (other viral infections and measles without secondary bacterial infections) should also be investigated.

**LITERATURE CITED**


