Peripheral T lymphocyte cytokine profile (IFN-γ, IL-2, IL-4) and CD30 expression/release during measles infection

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ABSTRACT

Background and Objective. Measles virus infection (MVI) has been reported to be characterized by an imbalanced Th1/2-type cytokine profile. CD30 has been proposed as a receptor preferentially associated with the Th0/2-type cytokine pattern. The aim of this study was therefore to define the peripheral T lymphocyte cytokine profile and to test which CD30 expression pattern it was associated with in MVI.

Design and Methods. The design of the study was a prospective evaluation with comparative analysis. The serum levels of the soluble form of CD30 (sCD30) were determined at diagnosis and at weekly intervals up to 4 weeks, using an ELISA, in 23 males (median age 19), who developed MVI while serving in the Italian army and who were admitted to the Infectious Disease Unit of the Military Hospital in Padua. In 10 of the patients at diagnosis we studied the lymphoid immunophenotype and, after nonspecific ex vivo stimulation, the expression of IFN-γ, IL-2 and IL-4 by peripheral T cells using flow cytometry single cell analysis. In 3 patients such evaluations were also performed 7 weeks later.

Results. At diagnosis, we found (i) reduction of IFN-γ+/CD4+ T cells (p<0.048 vs controls) in the absence of substantial variation of IL-2+ and IL-4+ T cells (p=ns vs controls); (ii) expansion of CD30+/CD4+ and CD30+/CD8+ T cell subsets (p<0.01 vs controls); (iii) high sCD30 values (median 61 U/mL; p<0.001 vs controls); (iii) a context of lymphopenia (0.728±0.292 lymph 3 10⁹/L). sCD30 remained elevated up to 4 weeks from MVI onset [median values 53, 49, 50, 34 U/mL after 1, 2, 3 and 4 weeks, respectively (p=ns between different time points)]. In 3 patients tested 7 weeks after diagnosis, we still observed decreased IFN-γ production by CD4+ and CD8+ T cells (p=0.05 and <0.01, respectively vs controls) and reduction of CD4+ and CD8+ IL-2+ T cells (p<0.01).

Interpretation and Conclusions. MVI was characterized by features of inadequate Th/Tc1 activation associated with increased circulating CD30+ T cells and elevated sCD30 levels, supporting a correlation between Th/ Tc status and CD30 expression/release pattern in vivo.

Key words: measles, cytokines, CD30, immune regulation, Th1/Th2 response

Measles virus infection (MVI) and, to some extent, vaccination against measles are accompanied by a number of immune abnormalities, including suppressed delayed-type hypersensitivity, defective mitogenic response of lymphocytes and impaired production of various cytokines in vivo and ex vivo. The clinical counterpart of such abnormalities in vivo is an acquired immunodeficiency, that can be regarded as a paradigm for other similar conditions and is arguably responsible for the secondary infections and autoimmune disorders that some individuals experience during or after MVI. Such complications may have serious clinical implications especially in developing countries and in immunocompromised hosts.

The immunity of MVI may probably be better understood on the basis of the Th1/2 paradigm which recognizes two polarized forms of CD4+ or CD8+ T cells, each preferentially associated with the cellular or the humoral arm of the immune system. CD4+ T-helper (Th) and CD8+ T cytotoxic (Tc) cells producing cytokines involved in classic cell-mediated functions, i.e. interferon (IFN)-γ, tumor necrosis factor-β and interleukin (IL)-2, are referred to as Th/TC1-type cells. T cells producing cytokines mainly involved in antibody responses, such as IL-4, IL-5, IL-10 and IL-13, are identified as Th/TC2-type cells. Th/TC0 cells produce both type 1 and 2 cytokines.

The overall pattern of immunologic abnormalities in MVI appears to be consistent with an imbalanced Th1/2-type immune polarization leading to a defective Th1- and to a prevalent Th2-type immune response. This immune pattern may be at least partly related to a down-regulation of IL-12, a major determinant of the switch to Th1-dominated responses, through virus-mediated inactivation of CD46 membrane protein.
The aim of our study was to characterize the immune response during MVI in a population of young adults by studying the intracellular cytokine profile (IFN-γ, IL-2, IL-4) and CD30 expression pattern in circulating T cells as well as levels of soluble CD30 (sCD30) in the blood. CD30 is a TNF receptor family cytokine receptor, which is involved in immune maturation and blood. CD30 is a TNF receptor family cytokine receptor whose expression is up-regulated by at least three signals: TCR triggering, IL-4 signaling through IL-4R and CD28 activation. The reason for investigating the CD30 molecule in MVI derives from previous observations suggesting that, following activation, CD30 is preferentially expressed and released by CD4+ and CD8+ Th/Tc clones, probably due to IL-4 activity on such cells. By contrast, Th/Tc clones only poorly and transiently express CD30. This supported a possible in vivo association between a Th/Tc shift and an increased number of CD30+ cells and/or elevated serum concentration of sCD30. Such an association, which is strongly suggested in some studies, is questioned in others. MVI is an additional condition in which to test the possible correlation between Th/Tc status and CD30 expression/release in vivo.

Design and Methods

Patients

We studied 23 young males (median age 19) who developed MVI while serving in the Italian army and were admitted to the Infectious Diseases Unit of the Military Hospital in Padua. The diagnosis was based on typical clinical symptoms and signs and supported by specific serologic and hematologic parameters. As a whole, the patients were monitored up to 7 weeks from diagnosis and/or until clinical normalization. Twenty-four healthy age-matched blood donors were used as normal controls.

Detection of serum levels of the sCD30 molecule

Sera were collected at diagnosis and 1, 2, 3 and 4 weeks after diagnosis in 23, 15, 11, 11 and 7 patients, respectively, and stored frozen at -70°C until use. They were investigated using a commercially available enzyme-linked immunosorbent assay for levels of sCD30 molecule [DAKO CD30 (Ki-1 antigen) ELISA, Dako, Glostrup, Denmark], as previously described. The mean (±SEM) serum level of sCD30 in 24 controls was 7.12±1.2, and the median level 5 U/mL.

Cell preparation and culture

Mononuclear cells from 10 patients at diagnosis, from 3 patients 7 weeks after diagnosis and from 11 healthy age-matched blood donors with no history of allergy and normal lymphocyte populations were aseptically separated from PB by Ficoll-Hypaque density gradient centrifugation and washed twice in 1% PBS solution. The mononuclear cells (2×10⁶/mL) were then resuspended in RPMI-1640 (GIBCO Laboratories, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal calf serum (FCS), 1% L-glutamine and antibiotics (penicillin plus streptomycin), and distributed in 24 well flat-bottomed plates for stimulation by PMA.

Cell stimulation

To be evaluated for cytokine production, mononuclear cells were stimulated with PMA (25 ng/mL) plus ION (1 µg/mL) for 4 hours at 37°C, 5% CO₂. After one hour, BFA (10 µg/mL) was added for the remaining three hours to block cytokine transport in Golgi's apparatus. Cellular suspensions were collected, centrifuged, washed twice in 1% PBS and resuspended at the final concentration of 10⁶ cells/mL.

Flow cytometry analysis

The following monoclonal antibodies (mAbs) were added to heparinized whole blood samples (100 µL) according to standard, previously described, procedures: fluorescein isothiocyanate (FITC)-conjugated anti-CD45, -CD3, -CD19, -CD8, -CD2, -CD45RA (Becton Dickinson, S. José, CA, USA) and -CD30 (Dako); phycoerythrin (PE)-conjugated anti-CD14, -CD5, -CD4, -CD8, -CD6, -CD36, -HLA-DR, and -CD122 (Becton Dickinson); tricolor-conjugated anti-CD3 (Caltag, South S. Francisco, CA, USA). Intracellular cytokine (IC) production was evaluated using tricolor-conjugated anti-CD4 and -CD8 (Caltag). After permeabilization (FACS solution, Becton Dickinson, 0.5 mL/sample for 10 minutes, room temperature), washing with 1%PBS/5%BSA/0.5% sodium azide, and incubation for 15 min with unconjugated mouse immunoglobulins (10 µg/10⁶ cells), FITC-conjugated anti-IFN-γ (Pharmingen, San Diego, CA, USA) and PE-conjugated anti-IL-2 (Pharmingen) and anti-IL-4 (Becton-Dickinson) mAbs were added to cells. After 30-minute incubation at 4°C, cells were washed and resuspended at 10⁶ cells/mL and analyzed with an argon-ion laser flow cytometer (FACScan, Becton Dickinson), using Cell Quest software. Adequate controls were performed. Lymphocyte surface markers and IC were evaluated at least 3×10⁴ and 5×10⁴ events, respectively. CD30 and IC were evaluated on the entire CD4+ and CD8bright+ lymphoid subsets.

Statistical analysis

Statistical comparisons included Kruskall-Wallis ANOVA by ranks, M ann-Whitney U-test or Student's t-test, according to the specific requisites of each set of data. Differences were considered statistically significant when the p value was <0.05.

Results

sCD30 values

We evaluated whether the serum levels of sCD30 were elevated or not during MVI. The results are shown in Table 1. Increased sCD30 levels were found in all cases but two in serum samples collected as soon as MVI was clinically recognizable (mean ±SEM: 96±20; median 61 U/mL) and sustained increased.

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values were observed up to one month from diagno-
sis. No significant difference was found when com-
paring sCD30 values at different time points. How-
ever, in 8 out of 11 patients who had a sequential
evaluation up to 3 weeks from diagnosis, elevated
sCD30 levels persisted throughout the observation
period, whereas 3 patients had sCD30 concentra-
tions which decreased progressively towards the
range of normal values (Figure 1).

Circulating lymphocyte immunophenotyping
We analyzed the absolute numbers and flow cytom-
etry pattern of lymphoid subsets at the time of diag-
nosis. Lymphopenia was present in all patients
(0.728±0.292 lymph ×10^9/L). Figure 2, panel A,
shows that the numbers of both CD4+ and CD8+-lym-
phocytes were reduced as compared to those in nor-
mal controls (0.199±0.110 and 0.144±0.090 vs
0.779±0.091 and 0.309±0.079 cells ×10^9/L, respec-
tively, p<0.05), with a ratio of 1.38±0.3. The immuno-
phenotype patterns of circulating lymphocytes in MVI
at diagnosis are listed in Table 2. B cells and NK cells
were not significantly increased. Among the CD3+-lym-
phocytes, CD45RA+ cells (31.23±9.09%, median
29.85%) were prevalent on CD45RO+ subset
(23.01±6.53%, median 23.90%). No significant
increase in CD25 (7.07±1.61%, median 7.00%) and
HLA-DR (13.71±8.77%, median 10.05%) expression
was detected as compared to controls (7.09±6.45%
and 10.46±4.06%, respectively), while CD122 was
expressed in a larger proportion of T cells in MVI than
in controls (8.95±6.99%, median 6.82% vs 2.0±1.3%).

CD30 expression on T cell subsets
We examined the expression of CD30 on T cell sub-
sets in detail. Figure 2, panel B shows that CD4+/CD30+
cells were similar to controls in terms of absolute numbers (p=0.66), while CD8+/CD30+ cells
tended to be increased, though not to a statistically
significant extent (p=0.11). In percentage terms, how-
ever, CD30+ cells were clearly expanded among both
CD4+ (5.88±2.26%, median 4.85% vs 1.6±0.6% in
controls) and CD8+ T cells (11.10±5.81%, median

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Table 1. Serum sCD30 values in measles virus infection.

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Median age (yrs)</th>
<th>sCD30 U/mL (mean±SEM)</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at diagnosis</td>
<td>23</td>
<td>19</td>
<td>96±20</td>
<td>61</td>
</tr>
<tr>
<td>1 week</td>
<td>15</td>
<td>17</td>
<td>67±18</td>
<td>53</td>
</tr>
<tr>
<td>2 weeks</td>
<td>11</td>
<td>18</td>
<td>55±18</td>
<td>49</td>
</tr>
<tr>
<td>3 weeks</td>
<td>11</td>
<td>17</td>
<td>52±10</td>
<td>50</td>
</tr>
<tr>
<td>4 weeks</td>
<td>7</td>
<td>20</td>
<td>61±30</td>
<td>34</td>
</tr>
<tr>
<td>Controls</td>
<td>24</td>
<td>22</td>
<td>7.12±1.2</td>
<td>5</td>
</tr>
</tbody>
</table>

Statistics: median sCD30 at different serum collection times: p=ns; MVI at
diagnosis vs controls: p<0.001.

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Figure 1. Time course evaluation of sCD30 serum concen-
tration in 11 patients with measles virus infection. Three
weeks after diagnosis, 8 patients had consistently high
sCD30 values, while 3 patients reverted to normal sCD30
values. Data are expressed as mean values (±SD) at each
given time. The shaded area corresponds to the range of
sCD30 values in normal controls.

Figure 2. Absolute number (mean±SD) of circulating CD4+, CD8+ and CD30+ T cells in 10 patients with measles virus
infection at diagnosis and in 11 controls. Panel A: CD4+ and
CD8+ T cells were significantly reduced as compared to con-
trols (p<0.05). Panel B: CD8+/CD30+ T cells showed a
tendency to increase without any statistical significance.
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10.2% vs 1.3±0.7%, p<0.01) in acute MVI (Table 2) and returned to normal values during the follow-up: circulating CD30+ cells were around 1% in all 3 cases evaluated at 7 weeks. A representative case is illustrated in Figure 3.

**Table 2. Immunophenotype analysis of circulating lymphocytes in measles virus infection at diagnosis**

<table>
<thead>
<tr>
<th></th>
<th>Measles virus infection</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean±SD</td>
<td>median</td>
</tr>
<tr>
<td>Lymph x10⁹/L</td>
<td>0.72±0.292</td>
<td>0.705</td>
</tr>
<tr>
<td>CD3+</td>
<td>52.61±10.68</td>
<td>54.45</td>
</tr>
<tr>
<td>CD3+/CD4+</td>
<td>27.43±9.42</td>
<td>30.15</td>
</tr>
<tr>
<td>CD3+/CD8+</td>
<td>19.81±7.52</td>
<td>21.15</td>
</tr>
<tr>
<td>CD3+/NALT+</td>
<td>13.71±8.77</td>
<td>10.05</td>
</tr>
<tr>
<td>CD3+/CD85</td>
<td>7.07±1.61</td>
<td>7.00</td>
</tr>
<tr>
<td>CD3+/CD122</td>
<td>8.95±6.99</td>
<td>6.82</td>
</tr>
<tr>
<td>CD4+/CD30+</td>
<td>5.8±2.62</td>
<td>4.85</td>
</tr>
<tr>
<td>CD8+/CD30+</td>
<td>11.10±8.81</td>
<td>10.20</td>
</tr>
<tr>
<td>CD4+/CD16-56-</td>
<td>4.3±3.4</td>
<td>3.00</td>
</tr>
<tr>
<td>CD8+/CD16-56-</td>
<td>19.47±15.73</td>
<td>12.75</td>
</tr>
<tr>
<td>CD4+/CD45RA+</td>
<td>2.3±1.65</td>
<td>2.50</td>
</tr>
<tr>
<td>CD4+/CD45R0+</td>
<td>19.93±9.08</td>
<td>18.90</td>
</tr>
<tr>
<td>CD4+/CD(16+56)+</td>
<td>19.47±15.73</td>
<td>12.75</td>
</tr>
<tr>
<td>CD4+/CD8+ ratio</td>
<td>1.38±0.3</td>
<td>1.42</td>
</tr>
</tbody>
</table>

**Table 3. Percentages of IFN\(\gamma\)+, IL-2+ and IL-4+ cells among circulating CD4+ and CD8+ T cells in measles virus infection at diagnosis.**

<table>
<thead>
<tr>
<th></th>
<th>Measles virus infection</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD4+</td>
<td>CD8+</td>
</tr>
<tr>
<td></td>
<td>mean±SD</td>
<td>median</td>
</tr>
<tr>
<td>IFN(\gamma)+</td>
<td>10.79±6.01</td>
<td>42.40±12.70</td>
</tr>
<tr>
<td>median</td>
<td>8.10</td>
<td>43.80</td>
</tr>
<tr>
<td>IL-2+</td>
<td>9.66±2.50</td>
<td>3.81±1.80</td>
</tr>
<tr>
<td>median</td>
<td>9.30</td>
<td>3.25</td>
</tr>
<tr>
<td>IL-4+</td>
<td>1.02±0.67</td>
<td>1.24±0.96</td>
</tr>
<tr>
<td>median</td>
<td>0.82</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Statistics: IFN\(\gamma\)+/CD4+ T cells in MVI vs controls, p=0.048; other percentages were not significantly different.

Discussion

The aim of our study was to investigate the possible relation between patterns of T cell cytokine production and CD30 expression as well as sCD30 serum concentrations in young adults with MVI. We found that acute MVI at diagnosis was characterized by: (i) low percentages of IFN\(\gamma\)+/CD4+ T cells; (ii) increased percentages of circulating CD4+/IFN\(\gamma\)+ and CD8+/IFN\(\gamma\)+ T cells; (iii) increased levels of serum sCD30; (iv) a context of lymphopenia, low expression of activation markers, and absence of a consistent switch from a naive (CD45RA+) to a memory (CD45R0) phenotype. During the follow-up, while circulating CD30+ T cells returned to within normal range, sCD30 values were persistently elevated up to 4 weeks after the acute phase of MVI, except in a minority of patients who reverted to normal sCD30 levels (Figure 1). In addition, IFN\(\gamma\) and IL-2 expression by T cells remained significantly decreased in all 3 patients evaluated 7 weeks after diagnosis.

These findings are consistent with a defective...
Th/Tc1-type response present at the beginning and full-blown in the late phases of MVI in association with increased sCD30 levels/circulating CD30+ T cells. Such a scenario is partly at variance with previous reports, suggesting a Th/Tc1-type cytokine pattern characterizing at least the acute phase of MVI. This diversity might be explained on technical grounds, such as the different experimental approach and/or different evaluation of normal controls, or on biological grounds, considering that we were evaluating a population consisting entirely of young adults.

In point of fact, the evidence argues against a Th/Tc1-type cytokine profile of circulating lymphocytes during the acute phase of MVI in our patients. We failed, however, to demonstrate any increase in IL-4+ T cells as compared to controls. By contrast, Ward and Griffin reported a predominant production of IL-4 after measles virus vaccination, suggesting induction of a Th2 response. Actually, IL-4, which, as a rule, is rapidly transported and does not accumulate to be easily demonstrated, is detectable in peripheral blood lymphocytes only following repeated T-cell stimulation in vitro or under conditions of long-lasting abnormal production in vivo. Thus, the lack of evidence of increased IL-4 expression by circulating lymphocytes in our MVI cases cannot be regarded as a definite argument against the possibility of a Th/Tc2-shift in MVI. Conversely, the clear-cut expression of both surface and soluble CD30 in MVI might suggest the production of amounts of IL-4, which is a major determinant of CD30+ lymphocyte appearance.

The deficient number of circulating cells producing Th/Tc1-type cytokines might actually reflect a Th/Tc0/2-type immune prevalence in lymphoid tissues, where viral replication is controlled mostly by the production of specific antibodies. This is even more likely because MV, through its interaction with CD46 membrane protein on infected cells, is directly responsible for down-regulating IL-12, a stimulatory factor inducing Th1-specific immune responses. In this context, the lack of the acquisition of a memory phenotype, as described above, suggests inadequate stimulation through IL-2, and, in fact, the percentage of IL-2+ cells in our MVI patients was similar to that observed in controls. On the whole, this would result in a state of anergy, explaining the suppressed delayed-type hypersensitivity, the defective mitogenic response of lymphocytes and the impaired production of various cytokines typically observed in MVI.

It may be of interest to remark that we found high percentages of circulating CD30+ T cells during the acute phase of MVI. This is not a usual finding in infectious or reactive diseases, even those characterized by high sCD30 values in the serum. Though there is agreement on the preferential expression of CD30 by Th/Tc2 clones, as previous evidence has suggested, CD30 expression may discriminate between Th/Tc1- and Th2-type cells has been questioned. As a matter of fact, CD30 knockout mice, which present a defect in negative selection, have normal Th2 differentiation and effector responses, indicating that CD30 is not required for Th2 development.

Nevertheless, as mentioned above, expansion of CD30+ subsets may be regarded as a hallmark of concomitant production of IL-4, even independently of its direct demonstration. Indeed, we have found high numbers of CD30+ T cells in the lymph nodes and skin in Omenn's syndrome, a Th2-dominated condition and similar results have been reported in other diseases characterized by strong, persistent activation of Th2 cells. By contrast, diseases dominated by activated T cells showing a...
Th/Tc1 cytokine profile have been usually reported to present no or rare CD30+ T cells,4,24-39 though a proportion of patients with diseases in which Th1 responses should be predominant have been demonstrated to present increased CD30+ cells and/or sCD30 levels.43-46 At least in some patients suffering from these diseases, however, production of both IL-4 and IFN-γ, i.e. a Th1/Tc0 pattern, can be postulated on the basis of preliminary experiments.

The present study characterizes MVI as an additional in vivo condition in which increased circulating CD30+ T cells and high sCD30 serum levels are detectable in association with a clear-cut impairment of Th/Tc1-type of cytokine pattern production. Clearly, only the demonstration that Th/Tc0-type cytokines are preferentially produced by CD30+ T cells in blood or tissues would definitely link CD30 expression and responses should be predominant have been demonstrated to present increased CD30+ cells and/or sCD30 levels.43-46 At least in some patients suffering from these diseases, however, production of both IL-4 and IFN-γ, i.e. a Th1/Tc0 pattern, can be postulated on the basis of preliminary experiments.

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Contributions and Acknowledgments
FV had main responsibility for all aspects of this study and for writing the paper. M K, LM, AR contributed to analysis of data and commented on the draft. SR and GP discussed core ideas and contributed to conception, design and critical revision of the paper. The order of authorship reflects these contributions.

The authors wish to thank Dr. Ezio Chinelli and Dr. Pietro Succurro, who were responsible for the care of patients at The Infectious Diseases Unit, M. I. University Hospital of Padua, for providing clinical data and biological samples.

Funding
This work was supported by grants from Associazione Italiana per la Ricerca sul Cancro (AIRC, Milano) and Progetto Nazionale Sanità 96/97, Fondazione Cariverona (Verona), Italy.

Disclosures
Conflict of interest: none.
Redundant publications: no substantial overlapping with previous papers.

Manuscript processing
A manuscript received February 8, 1999; accepted April 30, 1999.

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