Age-Related Expansion of Functionally Inefficient Cells With Markers of Natural Killer Activity in Down’s Syndrome

By Andrea Cossarizza, Claudio Ortolani, Ermenegildo Forti, Giuliano Montagnani, Roberto Paganelli, Maria Zannotti, Marina Marini, Daniela Monti, and Claudio Franceschi

Peripheral blood lymphocyte subsets of two groups of patients affected by Down’s syndrome (DS), ie, 28 children and nine adults of relatively advanced age (>34 years), were investigated and compared with those of age- and sex-matched healthy controls (13 children and 20 adults). Particular attention was devoted to cells with markers of natural killer (NK) activity. Double- and triple-color cytofluorimetric analysis was used to better characterize the phenotypic features of the different subsets. Apart from a reduced percentage of cells bearing markers associated with NK activity, such as CD16, CD56, and CD57. These DS cells were apparently severely defective as far as their function was concerned, because NK activity was significantly reduced in comparison with age-matched controls, but still capable of responding to cytokines such as interleukin-2, interferon-β, and interferon-γ, and to the modulation of lytic activity exerted by the anti-CD16 monoclonal antibody. On the whole, our data stress the importance of studying DS subjects of different ages to fully appreciate the immunologic derangement characteristic of this syndrome.

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Down’s Syndrome (DS) is the most frequent chromosomal aberration in human beings, characterized, in most cases, by an extra chromosome 21 (trisomy 21). In addition to mental retardation, DS patients show a derangement of the immune system that is thought to be responsible, at least in part, for the increased susceptibility to infectious diseases and could be involved in the increased frequency of leukemias. Indeed, infections are still among the major causes of death in DS patients. Moreover, DS patients show signs of precocious aging of various organs and tissues. According to some investigators, DS ranks first among human “segmental progeroid syndromes,” defined as those genetic disorders in which multiple major aspects of the senescent phenotype appear.

In the last decade, our studies have focused on the immune derangement and particularly on the precocious aging of the immune system in DS.

Age-related alterations of thymic hormone production, lymphocyte responsiveness to mitogens, micronuclei formation, and sensitivity to physical agents have been found.

In the present study we report a dramatic age-related expansion of cell subsets bearing markers related to natural killer (NK) activity in a total of 37 DS subjects whose age ranged from 2 to 62 years, in comparison with age- and sex-matched healthy controls. These cells were functionally ineffective but still capable of responding to several cytokines.

Materials and Methods

Subjects

The study was performed with the informed consent of the donors or of their parents.

A total of 37 subjects with karyotypically ascertained DS was studied, and all of them had a nontranslocated trisomy 21. They were subdivided into two groups: the first was composed of 28 children with a mean age of 9.2 ± 0.3 years (range, 2 to 14 years), the second by nine adults with a mean age of 44.2 ± 3.0 years (range, 34 to 62 years).

All of the DS children were noninstitutionalized and lived at home; six of nine DS adults were living in small institutes for handicapped people where the hygienic and sanitary conditions were optimal.

As control groups, 13 children with a mean age of 9.6 ± 0.6 years (range, 4 to 15 years) and 20 adults with a mean age of 45.0 ± 2.7 years (range, 21 to 60 years) were studied. In the great majority, control children were DS-patient kindreds, while control adults were healthy blood donors, laboratory personnel, and university professors.

Owing to shortage of cells, NK activity was studied in cells from 10 DS children (mean age 9.2 ± 1.2 years), seven DS adults (43.8 ± 3.5 years), 10 control children (10.5 ± 0.3 years), and 11 healthy adults (38.0 ± 2.5 years).

Hematologic Investigations

The total number of leucocytes was determined in all subjects by means of a Coulter counter (Coulter Electronics Inc, Hialeah, FL). The leucocyte differentiation count was performed by optic microscopy after staining with May-Grünwald following standard methods.

Phenotype Analysis and Flow Cytometry

Cells labeled with monoclonal antibodies (MoAbs) for cytofluorimetric analysis were prepared from whole blood following standard methods.

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Submitted February 15, 1990; accepted November 6, 1990.

Supported in part by C.N.R. (P.F. “Invecchiamento” and Progetto Strategico “Network per la raccolta di materiale biologico per studi di biologia molecolare delle malattie del sistema nervoso”), M.U.R.S.T. (40% and 60%), Sigma-Tau Foundation, and Sandoz Foundation for Gerontological Research grants to C. Franceschi. This work was done within the framework of European Concerted Action on Aging and Diseases (EURAGE). D.M. is supported by a Sigma-Tau Foundation fellowship.

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0006-4971/91/7706-0006$3.00/0
The following MoAbs from Becton Dickinson (Becton Dickinson Monoclonal Center, Mountain View, CA) directly conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE) were used to analyze the surface antigens of peripheral blood lymphocytes (PBL): anti-CD8 (anti-Leu2a), recognizing cytotoxic T-cell subset; anti-CD4 (anti-Leu3), reactive with helper/inducer T-cell subset; anti-CD3 (anti-Leu4), recognizing all T cells; anti-HLA-DR, recognizing B cells and activated T cells; anti-CD57 (anti-Leu7), reactive with a subset of cells with NK activity, with a subset of T lymphocytes, and with some CD8+ cells; anti-CD16 (anti-Leu11a or anti-Leu11c), recognizing the N-CAM molecule, reactive with resting and activated CD16+ cells, and with a small percentage of CD3+ lymphocytes, which are considered a subset of cytotoxic T lymphocytes that mediate non-major histocompatibility complex (MHC)-restricted cytotoxicity; anti-CD19 (anti-Leu12), reactive with B lymphocytes; and anti-CD14 (anti-Leu-M3), reactive with monocytes/macrophages, used to evaluate the percentage of monocytes present in the forward and orthogonal light scatter that were used to identify the lymphocyte gate during the cytofluorimetric analysis.14 IgG1, IgG2a, IgG2b, and IgM conjugated with FITC or PE (Becton Dickinson or Coulter Inc) were used as negative controls for nonspecific binding.

These cytofluorimetric analyses were performed with a FAC STAR cell sorter (Becton Dickinson). The FACSTAR was equipped with a 5-W argon laser operating at 300 mW at 488-nm wavelength to excite both FITC and PE fluorochromes.

A minimum of 10,000 cells per sample was analyzed. Regarding the value of the percentage of cells stained with each MoAb, for each subject the mean of all the determinations performed by the same MoAb conjugated with FITC or PE was calculated. PBL from five DS patients and five controls were also studied by using the three-fluorescence staining technique.

For the three-color immunofluorescence analysis, the MoAbs anti-CD57 (anti-Leu7), directly conjugated with FITC; anti-CD8 (anti-Leu2a), directly conjugated with biotin; and anti-CD16 or anti-CD56 (anti-Leu11c or anti-Leu19, respectively), directly conjugated with PE, were used. The third color was provided by Streptavidin-DuoCHROM (Becton Dickinson) detecting biotinylated MoAbs. The analysis was performed on a FACScan (Becton Dickinson), and the parameter “time” has been taken as an internal control of the data acquisition to avoid false electronic signals related to flow troubles.15 On the FACScan, detector and compensation levels were set using, firstly, CaliBRITE beads in conjunction with the AutoCOMP software and then, secondly, PBL stained with anti-CD19 (Leu12-FITC), anti-CD8 (Leu2a-PE), and anti-CD4 (Leu3-Biotin-DuoCHROM). Data were acquired in list mode by FACScan Research software and analyzed by the Paint-A-Gate software program.

Cytotoxicity Assays

Mononuclear cells were separated from venous blood following standard methods, and monocytes removed by incubating the mononuclear cell suspension in a Petri dish for 1 hour at 37°C. Cytotoxicity against K562 cells, a human erythroleukemia cell line, was determined in a 4-hour 51Cr-release assay.16 Target cells, free of mycoplasma, were labeled overnight with sodium 51Cr solution (Amersham Int, Amersham, UK) in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 2 mmol/L glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. The effecter/target ratio varied from 100:1 to 3:1 depending on the effector population tested. All experiments were performed in quadruplicate. The percentage of specific cytotoxicity was calculated according to the following formula:

\[
\text{specific cytotoxicity} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{maximal release} - \text{spontaneous release}} \times 100
\]

The mean spontaneous release from the medium control never exceeded 15%.

Data on NK cytotoxicity are expressed in terms of lytic units (LUₜₐₚ)×10⁶ cells, calculated according to Pross et al.19 on the basis of the dose-response curve. One LUₜₐₚ corresponds to the number of effector cells necessary to lyse 30% of the targets.

In some experiments, before the cytotoxicity test, cells from adult DS subjects and from healthy controls were incubated for 18 hours with 40 U/mL human recombinant interleukin-2 (IL-2; Boehringer, Mannheim, Germany), or with 1,000 U/mL human recombinant interferon (IFN)-γ (Shionogi Pharmaceutical Co, Kyoto, Japan), or 1,000 U/mL human recombinant IFN-β (Shionogi) to evaluate the capability of cytotoxic cells to be modulated by different cytokines.

The effect of the presence of an anti-CD16 MoAb (anti-Leu1c, which is an IgG1) in the medium during the cytotoxic assay was also investigated. As control, an anti-HLA-DR MoAb was used. The final concentration of the MoAbs was 0.1 μg/mL. PBL from four DS adults and from three healthy controls were studied, and data are expressed as percentage of specific cytotoxicity.

Statistical Analysis

Statistical analyses were performed by two-tail Student’s t-test and by the linear regression analysis.

RESULTS

Phenotypic Analysis of PBL

Single-fluorescence staining. Table 1 shows that significant alterations were present in DS subjects as far as the percentage and the absolute number of several PBL subsets are concerned. DS children, but not DS adults, presented a decreased number of circulating lymphocytes/mm², in accord with previous studies from our group.10,12

The percentage of CD3+ PBL was similar in all the groups; a significant reduction of T cell number was present in DS children, but not in DS adults.

A marked reduction of the percentage and the number of CD4+ cells was present in DS children in comparison with age-matched controls. In DS children the percentage of CD8+ lymphocytes increased, but the absolute number of these cells was comparable with that of normal controls. In DS adults both the percentage and the absolute number of CD8+ cells were significantly augmented.

A significant reduction of B cells (CD19+) was observed in both groups of DS subjects.

As far as cells with phenotypes related to NK activity were concerned, three groups of cells were considered. CD57+ lymphocyte percentage increased with age in control subjects, as previously reported,20 but this phenomenon was much more evident in DS subjects. Both the percentage and the number of these cells were dramatically increased in DS adults.
ALTERATIONS OF NK SYSTEM IN DOWN'S SYNDROME

Table 1. Phenotypic Analysis of PBL From the Subjects Studied

<table>
<thead>
<tr>
<th></th>
<th>DS Children</th>
<th>Control</th>
<th>DS Adults</th>
<th>Control</th>
<th>Statistical Analysis</th>
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<tr>
<td></td>
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<tr>
<td><strong>Lymphocytes</strong></td>
<td></td>
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<tr>
<td><strong>CD4</strong>%</td>
<td>33.4 ± 3.1</td>
<td>43.1 ± 3.1</td>
<td>29.5 ± 2.9</td>
<td>46.2 ± 1.6</td>
<td>&lt;.02 &lt;.001 NS NS</td>
</tr>
<tr>
<td><strong>CD8</strong>%</td>
<td>16.3 ± 2.4</td>
<td>71.6 ± 3.3</td>
<td>71.9 ± 2.7</td>
<td>72.0 ± 1.6</td>
<td>NS NS NS NS</td>
</tr>
<tr>
<td><strong>CD57</strong>%</td>
<td>36.7 ± 1.7</td>
<td>27.3 ± 1.9</td>
<td>44.5 ± 1.5</td>
<td>27.3 ± 1.5</td>
<td>&lt;.005 &lt;.001 &lt;.02 NS</td>
</tr>
<tr>
<td><strong>CD56</strong>%</td>
<td>7.3 ± 0.7</td>
<td>11.8 ± 1.6</td>
<td>5.3 ± 1.1</td>
<td>9.6 ± 0.7</td>
<td>&lt;.005 &lt;.001 NS NS</td>
</tr>
<tr>
<td><strong>NK/lymphocytes</strong></td>
<td>4.2 ± 0.4</td>
<td>1.6 ± 0.5</td>
<td>7.0 ± 1.2</td>
<td>3.7 ± 0.4</td>
<td>&lt;.005 &lt;.001 &lt;.004 NS</td>
</tr>
<tr>
<td><strong>CD16</strong>%</td>
<td>6.8 ± 0.9</td>
<td>7.7 ± 1.5</td>
<td>10.9 ± 2.1</td>
<td>8.2 ± 1.4</td>
<td>NS NS &lt;.05 NS</td>
</tr>
<tr>
<td><strong>CD57</strong>, CD16+</td>
<td>15.0 ± 1.7</td>
<td>8.8 ± 1.4</td>
<td>32.3 ± 3.4</td>
<td>14.5 ± 1.3</td>
<td>&lt;.05 &lt;.001 &lt;.001 &lt;.001 NS</td>
</tr>
<tr>
<td><strong>CD56</strong>, CD8+</td>
<td>334 ± 48</td>
<td>291 ± 77</td>
<td>717 ± 82</td>
<td>316 ± 28</td>
<td>NS &lt;.001 &lt;.001 NS</td>
</tr>
</tbody>
</table>

Data are referred to the percentage (%) or to the absolute number/mm³ (n) of PBL positive to the MoAbs used. Statistical analysis was performed by Student’s t-test, between DS children and control children (A), DS adults and control adults (B), DS children and DS adults (C), and control children and control adults (D).

Abbreviations: T-act, T-activated lymphocytes (CD3+, HLA DR+); NS, not significant.

No significant differences in the percentage and number of CD16+ cells were observed in both DS children and DS adults. The percentage of CD56+ lymphocytes was higher in DS children than in control children, but the absolute number did not present a significant increase. Both these parameters were markedly augmented in DS adults.

A significant increase of CD3+, HLA DR+ cells, ie, T-activated lymphocytes, was present in DS children and adults.

In all groups, the percentage of monocytes in the lymphocyte gate was less than 0.8% (data not shown).

**Double-fluorescence staining.** The use of double-fluorescence staining allowed us to perform a more detailed analysis of cells with NK-related phenotypes (Table 2).

Table 2. Double-Staining Analysis of Cells With NK Markers

<table>
<thead>
<tr>
<th></th>
<th>DS Children</th>
<th>Control</th>
<th>DS Adults</th>
<th>Control</th>
<th>Statistical Analysis</th>
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<tbody>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>CD57+, CD16−</strong></td>
<td>18.3 ± 2.0</td>
<td>5.3 ± 0.9</td>
<td>35.5 ± 4.5</td>
<td>12.1 ± 2.0</td>
<td>&lt;.01 &lt;.001 &lt;.001 &lt;.02</td>
</tr>
<tr>
<td><strong>CD57+, CD16+</strong></td>
<td>26.0 ± 4.4</td>
<td>172 ± 36</td>
<td>944 ± 186</td>
<td>264 ± 44</td>
<td>&lt;.02 &lt;.001 NS NS</td>
</tr>
<tr>
<td><strong>CD57−, CD16+</strong></td>
<td>2.6 ± 0.4</td>
<td>2.3 ± 0.5</td>
<td>7.2 ± 1.7</td>
<td>2.9 ± 0.6</td>
<td>NS &lt;.001 &lt;.001 NS</td>
</tr>
<tr>
<td><strong>CD57−, CD16−</strong></td>
<td>55 ± 9</td>
<td>78 ± 22</td>
<td>194 ± 47</td>
<td>41 ± 13</td>
<td>&lt;.001 &lt;.001 NS NS</td>
</tr>
<tr>
<td><strong>CD57−, CD8+</strong></td>
<td>110 ± 1.4</td>
<td>3.0 ± 0.9</td>
<td>24.1 ± 3.6</td>
<td>8.9 ± 1.3</td>
<td>&lt;.005 &lt;.001 &lt;.003 &lt;.003 NS</td>
</tr>
<tr>
<td><strong>CD57+, CD4−</strong></td>
<td>2.0 ± 0.4</td>
<td>0.6 ± 0.2</td>
<td>2.5 ± 0.4</td>
<td>ND</td>
<td>&lt;.05 ND NS NS NS</td>
</tr>
<tr>
<td><strong>CD57+, CD56−</strong></td>
<td>36 ± 0.5</td>
<td>2.2 ± 0.6</td>
<td>9.5 ± 1.4</td>
<td>ND</td>
<td>NS ND ND ND</td>
</tr>
<tr>
<td><strong>CD16+, CD8−</strong></td>
<td>2.8 ± 0.4</td>
<td>2.4 ± 0.4</td>
<td>5.3 ± 0.5</td>
<td>ND</td>
<td>NS &lt;.003 ND ND</td>
</tr>
<tr>
<td><strong>CD16−, CD56−</strong></td>
<td>32 ± 0.7</td>
<td>5.4 ± 1.7</td>
<td>9.8 ± 1.2</td>
<td>4.8 ± 0.9</td>
<td>NS &lt;.001 &lt;.001 NS</td>
</tr>
<tr>
<td><strong>CD56−, CD8+</strong></td>
<td>53 ± 1.0</td>
<td>2.6 ± 0.5</td>
<td>11.1 ± 1.3</td>
<td>ND</td>
<td>NS ND &lt;.005 ND</td>
</tr>
<tr>
<td><strong>CD3+, CD56−</strong></td>
<td>3.4 ± 0.8</td>
<td>0.8 ± 0.2</td>
<td>8.8 ± 1.9</td>
<td>1.4 ± 0.5</td>
<td>&lt;.02 &lt;.001 &lt;.001 NS</td>
</tr>
<tr>
<td><strong>CD3−, CD56+</strong></td>
<td>76 ± 13</td>
<td>27 ± 9</td>
<td>209 ± 46</td>
<td>28 ± 7</td>
<td>&lt;.05 &lt;.001 &lt;.001 NS</td>
</tr>
</tbody>
</table>

Data refer to the percentage (%) or to the absolute number/mm³ (n) of PBL positive to one or both the MoAbs used. Statistical analysis was performed by Student’s t-test, between DS children and control children (A), DS adults and control adults (B), DS children and DS adults (C), and control children and control adults (D).

Abbreviations: NS, not significant; ND, not determined.
Fig 1. Three-color cytofluorimetric analysis of PBL from a 55-year-old DS patient that is representative of the group of DS adults.
The staining with anti-CD57 and anti-CD16 MoAbs distinguished three populations with different NK activity, ie, CD57+, CD16- cells, CD57+, CD16+ cells, and CD57-, CD16+ cells, capable of low, intermediate, and high NK activity, respectively.*

Both groups of DS patients showed a significant increase of cells with low NK activity. DS adults also showed an increase of cells with intermediate NK activity. Interestingly, the number of CD16+, CD57- lymphocytes was markedly diminished in DS children but not in DS adults.

Another cell subset with consistent NK activity, ie, CD56+, CD16+ cells, was decreased in DS children but increased in DS adults.

CD3+, CD56+ lymphocytes, mediating non-MHC-restricted cytotoxicity, were increased in both DS groups.

One of the most relevant alterations of T-lymphocyte subsets in DS was the marked and significant expansion of CD8+, CD57+ cells, ie, a subset mainly composed of MHC-restricted cytotoxic T lymphocytes, present in both DS children and adults.

Other CD8+ cells, such as CD8+, CD16+ lymphocytes, a heterogeneous not well-defined subset comprising cells capable of MHC-unrestricted cytotoxicity, were not augmented in DS children.

**Triple-fluorescence staining.** To better characterize the most expanded subpopulations of cells in DS adults, ie, CD57+, CD16- and CD57+, CD8+ subsets, cells from five relatively aged DS patients and five control adults were analyzed by a panel of four MoAbs by three-color cytofluorimetric analysis.

In Fig 1 and Table 3, a representative example taken from a 55-year-old DS patient, in which a striking derangement of cellular subsets was observed, is reported and compared with an age-matched healthy control. In all of the five relatively aged DS patients and five control adults were analyzed by a panel of four MoAbs by three-color cytofluorimetric analysis.

As expected, in normal subjects a positive correlation was observed between natural cytotoxic capacity and frequency of CD16+ and CD56+ cells, and in particular of CD16+, CD57- and CD16+, CD56+ subsets. On the contrary, no correlation between any NK phenotype and activity was observed in DS subjects.

Table 4. NK Activity of PBL From the Subjects Studied

<table>
<thead>
<tr>
<th></th>
<th>Children</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS (n = 10)</td>
<td>Control (10)</td>
<td>DS (7)</td>
</tr>
<tr>
<td>LU&lt;sub&gt;10&lt;/sub&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.3 ± 9.1</td>
<td>90.5 ± 10.8</td>
<td>31.6 ± 4.31</td>
</tr>
</tbody>
</table>

Data are expressed as LU<sub>10</sub>/10<sup>7</sup> cells (mean ± SE) according to the formula quoted in Materials and Methods. Statistical analysis was performed by Student's t-test. The comparisons between DS children and DS adults and between control children and control adults showed no statistical differences.

In this study we confirmed the presence in DS children of a significant reduction of T lymphocyte absolute number, and particularly of CD4+ cells, likely related to the thymic dysfunction characteristic of the syndrome.4,10,12,21

As CD8+ cells did not show a concomitant decrease, an inverted CD4+/CD8+ lymphocyte ratio was evident in DS children. This phenomenon was even more pronounced in

DISCUSSION

In this study we confirmed the presence in DS children of a significant reduction of T lymphocyte absolute number, and particularly of CD4+ cells, likely related to the thymic dysfunction characteristic of the syndrome.4,10,12,21

As CD8+ cells did not show a concomitant decrease, an inverted CD4+/CD8+ lymphocyte ratio was evident in DS children. This phenomenon was even more pronounced in
Table 5. Modulation of NK Activity in DS Patients

<table>
<thead>
<tr>
<th>E/T Ratio</th>
<th>DS Subjects</th>
<th>Healthy Adults</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Age (y)</td>
<td>51</td>
<td>47</td>
</tr>
<tr>
<td>Control</td>
<td>100:1</td>
<td>38.9</td>
</tr>
<tr>
<td>50:1</td>
<td>33.4</td>
<td>23.3</td>
</tr>
<tr>
<td>+ IL-2</td>
<td>100:1</td>
<td>81.5</td>
</tr>
<tr>
<td>50:1</td>
<td>82.3</td>
<td>82.4</td>
</tr>
<tr>
<td>+ IFN-γ</td>
<td>100:1</td>
<td>74.5</td>
</tr>
<tr>
<td>50:1</td>
<td>74.0</td>
<td>77.8</td>
</tr>
<tr>
<td>+ IFN-β</td>
<td>100:1</td>
<td>80.4</td>
</tr>
<tr>
<td>50:1</td>
<td>77.2</td>
<td>79.9</td>
</tr>
<tr>
<td>+ anti-CD16</td>
<td>100:1</td>
<td>56.4</td>
</tr>
<tr>
<td>50:1</td>
<td>49.0</td>
<td>60.9</td>
</tr>
</tbody>
</table>

PEL were incubated for 18 hours with human recombinant IL-2 (40 U/mL), IFN-γ (1,000 U/mL), or IFN-β (1,000 U/mL) before the assay. Anti-CD16 MoAb (anti-Leu1 IC, 0.1 µg/mL) was added immediately before the 4 hours incubation with K562 target cells. Data are expressed as percentage of specific lysis.

Abbreviation: ND, not determined.

DS adults, where a marked increase of CD8+ cell percentage and absolute number was present. Thus, an inverted CD4+/CD8+ lymphocyte ratio appears to be characteristic of DS.24 The decreased absolute number of B lymphocytes we observed in both groups of DS patients is in accord with previous observations.25 This phenomenon, despite its consistency, has received little attention. Indeed, alterations of Ig class and subclass plasma levels have been reported in DS children,25 but the relationships among the reduction of circulating B cells, susceptibility to infections, and Ig derangements are far from being clear.

Besides the above mentioned alteration of T and B lymphocytes, DS subjects showed characteristic alterations of cells with NK markers and decreased NK activity.

In DS, a relevant age-related expansion of CD57+ cells was observed. This subset increases during physiologic aging,20 but in DS this phenomenon was accelerated and augmented.

CD57+ lymphocytes can be subdivided into several subsets. A marked increase of CD57+, CD8+ cells was observed in DS children and adults. These cells are augmented during chronic activations of the immune system, such as viral infection, autoimmune diseases, and chronic graft-versus-host disease, among others.26 The increase of this subset could account, at least in part, for the increased percentage of CD8+ cells observed in DS adults. Moreover, these data suggest the presence in DS of an activation of the immune system of unknown nature. Indeed, a significant increase of T-activated lymphocytes was observed in DS children and adults. Other CD57+ cell subset are represented by CD57+, CD16− and CD57+, CD16+ cells, characterized by low and intermediate NK activity, respectively.21 In DS patients, only cells with low NK activity were expanded, and this phenomenon was particularly evident in DS adults, where they reached more than one third of all PBL. Cells with intermediate NK activity were also increased, but to a lower extent and only in DS adults.

On the other hand, cells with a phenotype related to a high NK activity, ie, CD57−, CD16+ lymphocytes, were decreased in DS children and unaffected in DS adults. In both DS groups a low NK activity, together with a lack of correlation between the percentage of such cells and NK activity, was observed. This fact, together with the finding that a similar lack of correlation was found for another subset with high NK activity, ie, CD16+, CD56+ cells, suggests that functional defects of subsets responsible for most NK activity are present in DS concomitantly with an expansion of cells with low cytotoxic capacity.

Table 6. Correlation Between NK Activity and NK Phenotypes in the Groups Studied

<table>
<thead>
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<th>Children</th>
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<th>Adults</th>
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<tr>
<td></td>
<td>DS</td>
<td>Control</td>
<td>DS</td>
<td>Control</td>
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</tr>
<tr>
<td>CD16+</td>
<td>r</td>
<td>- .330</td>
<td>.881</td>
<td>- .377</td>
<td>.910</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>NS</td>
<td>&lt; .005</td>
<td>NS</td>
<td>&lt; .001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD56+</td>
<td>r</td>
<td>.819</td>
<td>.847</td>
<td>- .471</td>
<td>.889</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>NS</td>
<td>&lt; .008</td>
<td>NS</td>
<td>&lt; .001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD16+, CD57−</td>
<td>r</td>
<td>- .078</td>
<td>.965</td>
<td>- .226</td>
<td>.891</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>NS</td>
<td>&lt; .001</td>
<td>NS</td>
<td>&lt; .001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD16+, CD56+</td>
<td>r</td>
<td>- .228</td>
<td>.877</td>
<td>- .451</td>
<td>.816</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>NS</td>
<td>&lt; .005</td>
<td>NS</td>
<td>&lt; .001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The natural cytotoxic capacity of PBL from each subject (expressed in LU/L/10⁸ cells) was correlated by the linear regression analysis to his/her percentage of cells bearing an NK marker. The markers analyzed were all of those reported in Tables 1 and 2, but in this table only the positive correlations are reported. A statistically significant result indicates that into the group a positive correlation between the percentage of cells with a marker related to NK activity and the natural cytotoxicity was present.

Abbreviations: r, correlation index; P, statistical significance.
A very small subset of cytotoxic cells, ie, CD3+, CD56+ lymphocytes, are increased in both the DS groups. They are considered T cells capable of non-MHC-restricted cytotoxicity, and appear to play an important role as tumor-infiltrating lymphocytes. The expansion of this small subset could be the consequence of an effort of DS immune system to compensate for the functional and numerical reduction of other cytotoxic subsets. The hypothesis may be put forward that the thymic hypoplasia present in DS, leading to a reduction of thymic hormone production, would favour the differentiation of cells that share characteristics of both T (CD3 complex associated to the T-cell receptor) and NK cells (non-MHC-restricted cytotoxic activity and CD56 molecule).

Three-color cytofluorimetric analysis of cells with markers of NK activity allowed us to demonstrate that the highly expanded subsets of CD57+, CD16 and of CD57+, CD8+ cells are heterogeneous and likely not clonal, being constituted by CD56+ or CD56− cells and by CD16+ or CD16− cells, respectively.

Previous observations of other authors who studied cells with NK activity markers in a less detailed manner are mostly in agreement with our observations. The nature of the defect of NK function in DS is not clear. However, this defective lytic capability was fully restored in DS adults by preincubation with either IL-2 or IFN-β and IFN-γ. The mechanism of upregulation of NK activity by these cytokines is controversial. Cytokines could augment the function of pre-existing NK cells by increasing the recycling, or recruiting NK precursors. The defective NK function of DS cells was also increased by the addition of anti-CD16 MoAb during the 4-hour cytotoxic test. Several mechanisms can be hypothesized to account for this phenomenon, ie, activation of effector cells via low-affinity FcγG receptor triggered by the MoAb, increased effector-target contact, and triggering of an antibody-dependent cell-mediated cytotoxicity (direct or reversed) via FcγG receptor present on K562 cells.

In any case, these data indicate that alterations of the lytic machinery of DS cells can be excluded.

A critical event of cytotoxic reactions is cell-cell interaction. Indeed, defects of cell-adhesion molecules coded by genes mapping on chromosome 21 have been described in DS. The exogenous addition of cytokines and of MoAb anti-CD16 likely bypasses the critical step of effector-target adhesion.

In conclusion, in DS a complex age-related impairment of T, B, and NK cell production, differentiation, and activity takes place. The sum of these alterations is likely the cellular basis of the defective immune responses and of the increased susceptibility to infectious diseases occurring in DS patients. It is not clear whether these alterations are the consequence or the cause of the process of accelerated aging characteristic of the syndrome.

ACKNOWLEDGMENT

We thank Dr M. Londei, Dr P. Allavena, Dr M.C. Sirianni, and Prof M. Fiorilli for helpful discussions; Sisters Gemma, Adelia, and Riccarda (Suore della Divina Volonta’, Bassano del Grappa), M.T. Flumiani, and Dr C. Cavazzuti for their help in blood collection. The families of the DS patients are also gratefully acknowledged.

REFERENCES

pulsed electromagnetic fields as a biomarker of precocious aging in Down's syndrome. Aging (in press)


32. Fanger MW, Shen L, Graziano RF, Guyre PM: Cytotoxicity mediated by human Fc receptors for IgG. Immunol Today 10:92, 1989

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