Activation and Increased Expression of Adhesion Molecules on Peripheral Blood Lymphocytes Is a Mechanism for the Immediate Lymphocytopenia After Administration of OKT3

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We investigated the mechanism by which antihuman CD3 monoclonal antibodies of the isotypes IgG2a (eg, OKT3) and IgA (eg, IXA) can induce the rapid disappearance of virtually all circulating T lymphocytes. We hypothesize that upregulation of adhesion molecules on the lymphocyte membrane contributes to this effect. However, this hypothesis is difficult to test, because of the inherent lymphocytopenia and/or shifts in lymphocyte populations between intra- and extravascular compartments. Therefore, studies in vitro were performed, as well. Analysis of peripheral blood lymphocytes isolated at several times after addition of OKT3 or IXA to whole blood of healthy individuals showed an immediate increase in the proportion of T cells expressing NKI-L16, an activation epitope on CD11a/CD18. Likewise, an increase in CD11b/CD18 expression occurred. In parallel experiments, a transiently increased adhesion of T cells to endothelial cell monolayers was observed. This adhesion could be completely blocked by anti-CD18 or anti-CD11a monoclonal antibodies and only partly by an anti-CD11b antibody. Our data indicate that upregulation of activation epitopes of CD11a/CD18, as well as increased expression of CD11b/CD18 on T lymphocytes, may result in increased adhesion of these cells to intercellular adhesion molecule-1 (ICAM-1) and ICAM-2 on vascular endothelium. This phenomenon may, at least, partly explain the rapidly occurring peripheral lymphocytopenia observed in vivo.

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MATERIALS AND METHODS

Methods

The study was approved by the institutional research and ethical committees of the Academic Medical Center of the University of Amsterdam. Written informed consent was obtained from all patients.

In Vivo Studies

Patients. Ten renal transplant recipients (three men, seven women, median age 31 years, range, 20 to 61 years) were studied. These patients were treated for 10 consecutive days with 5 mg/day OKT3 (Ortho Diagnostic Systems, Raritan, NJ) because of acute kidney allograft rejection, diagnosed on the basis of clinical manifestations and confirmed by core biopsy. As recommended by the manufacturer, before OKT3 administration on day 1, a single dose of 500 mg methylprednisolone was given. The control group consisted of 10 renal transplant recipients (four men, six women, median age 31 years, range, 21 to 54 years) who were treated for acute rejection with methylprednisolone 500 mg/day for 6 consecutive days. Basic immunosuppressive therapy in both groups consisted of prednisolone (10 mg/day) and cyclosporin (dosage adjusted so as to result in whole-blood trough levels of 150 to 200 μg/L).

Materials and methods. Peripheral blood was collected before the administration of 500 mg methylprednisolone and at 3, 10, 15, 30, 60, 120 minutes, 4.5, and 24 hours after administration of either 5 mL (ie, 5 mg) OKT3 or 5 mL 0.9% saline (control group). At each time point, total leukocyte and differential counts were determined by flow cytometry (Technicon H1 System; Bayer/Technicon, Tarrytown, NY) on blood anticoagulated with 0.38 mmol/L EDTA, pH 7.4. OKT3 was introduced into whole-blood samples within 2 hours of collection by two washings of the mononuclear cells in PBS-A (ie phosphate buffered saline (PBS) containing 0.5% wt/vol bovine serum albumin and 0.02% wt/vol sodium azide).

Peripheral blood mononuclear cells were isolated by centrifugation of heparinized blood on Ficoll-Paque (density 1.076; Pharmacia, Uppsala, Sweden). Contaminating erythrocytes were lysed by incubation with ammoniumchloride (155 mM NH₄Cl, 10 mM KHCO₃, 0.1 mM EDTA, pH 7.4) for 10 minutes on ice, followed by two washings of the mononuclear cells in PBS-A (ie phosphate buffered saline (PBS) containing 0.5% wt/vol bovine serum albumin (Rosera DEM, Organon Teknika B.V., Boxtel, Holland) followed by a wash in Tris-buffered Earle’s Balanced Salt Solution containing 1% fetal calf serum).

Immunofluorescence. Peripheral blood mononuclear cells were incubated for 30 minutes at 0°C in PBS-A with appropriate dilutions of the fluorescein isothiocyanate (FITC)-labeled monoclonal antibodies CD3 (Dako, Glostrup, Denmark, clone HM24, IgG1k), NKL-16 (Southern Biotechnology, Birmingham, AL), and CD16 (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (CLB), Amsterdam, The Netherlands; clone CLB-149, IgG2a), and with the phycoerythrin-labeled monoclonal antibody CD11b (Dako; clone 2F1.21, IgG1k). For determination of CD62L and CD49d/CD29 expression, an indirect staining procedure was used consisting of primary incubation of the mononuclear cells with appropriate dilutions of the monoclonal antibodies LECAM-1 (directed against CD62L, Becton Dickinson, Leu-2a, IgG2a), and with phycoerythrin-labeled streptavidin (Becton Dickinson) or FITC-labeled goat antimouse IgG1 (Southern Biotechnology, Birmingham, AL), respectively. All samples were two-color stained with a phycoerythrin- or FITC-labeled anti-CD2 monoclonal antibody (Dako; clone MT79/10, IgG1k or Becton Dickinson, Leu-5b; clone S5.2, IgG2a, respectively) and with a phycoerythrin- or FITC-labeled anti-CD3 monoclonal antibody (both from Becton Dickinson, Leu-4; clone SK7, IgG1) and (2) with FITC labeled F(ab′)₂ fragments of goat antimouse Ig (GAM F(ab′)₂, obtained from the CLB. Analysis of cell suspensions was performed on a FACScan (Becton Dickinson). Lymphocytes in the mononuclear cell population were gated on forward and side scatter.

In Vitro Studies

Immunofluorescence. A total of 55 mL heparinized peripheral blood was taken from five healthy individuals and kept in a 37°C waterbath. A 5-mL sample was set apart and the remaining 50 mL blood was divided into four separate portions, to which OKT3, IXA, an irrelevant murine monoclonal antibody of the IgG2a isotype (P23-49, directed against Mycobacterium tuberculosis) or an irrelevant murine monoclonal antibody of the IgA isotype (clone TEC15, mouse IgAκ myeloma protein; Sigma, St Louis, MO) was added, each at a final concentration of 1 μg/mL, which is comparable to serum levels after administration of 5 mg OKT3 in vivo. At 3, 30, and 60 minutes after addition of the monoclonal antibody, 3 mL samples were taken. Peripheral blood mononuclear cells were separated by Ficoll-Paque density gradient centrifugation and expression of the same adhesion molecules as analyzed in the in vivo studies was measured on a FACScan as described above. At each time point, total leukocyte and differential counts were determined by flow cytometry (Technicon H1 System).

Functional Adhesion Studies

From eight healthy individuals, 40 mL heparinized peripheral blood was taken. Mononuclear cells were isolated by centrifugation on Ficoll-Paque, followed by three washings in Tris-buffered Earle’s Balanced Salt Solution containing 5% fetal calf serum. Next, CD3+ T cells were obtained by a negative selection procedure: removal of B lymphocytes, monocytes, and natural killer cells by incubation with murine monoclonal antibodies against CD14 (CLB, clone 11G1), anti-CD14 (CLB, clone 8G3), and anti-CD16 (CLB, gran-1) followed by incubation with sheep antimouse IgG coated Dynabeads (Dynal A.S., N-0212, Oslo, Norway). This procedure resulted in >95% pure T-cell suspensions as confirmed by fluorescence-activated cell sorter (FACS) analysis after staining the cells with a mixture of FITC-labeled CD3- and phycoerythrin-labeled CD16 and CD56 monoclonal antibodies (NK-Simulset, Becton Dickinson). The CD3-positive cells (2 × 10⁶ cells/mL) were incubated on ice for 30 minutes with OKT3, IXA, the irrelevant IgG2a monoclonal antibody P23-49, or the irrelevant IgA monoclonal antibody TEP1C15, each at a final concentration of 1 μg/mL, followed by two washings at 4°C with Iscove’s modified Dulbecco’s medium containing 5% fetal calf serum. Next, 2 μg/mL goat antimouse Ig (GM17D, CLB) was added to cross-link the T-cell receptor/CD3 complexes, immediately followed by 3 minutes 10% centrifugation at 37°C. Part of the cell suspension was then added to a cell monolayer consisting of an immortalized endothelial cell line derived from human umbilical vein endothelial cells, cultured in 96-well plates (Nunclon Micro Well P96, Nunc A.S., Roskilde, Denmark) on 1% gelatin (Sigma, G1890) in PBS. The remaining cell suspension was placed in a shaking waterbath at 37°C and after 30 and 60 minutes of incubation, samples were added to the endothelial cell monolayers. The 96-well plates were incubated for 15 minutes at 37°C in 5% CO₂, followed by removal of the nonadherent fraction by washing three times with 200 μL 37°C Earle’s Balanced Salt Solution containing 5% fetal calf serum. The adherent fraction was washed with Diff Quick (a 3-step staining procedure comprising: (1) fixation in methanol with fast green 0.002 g/L, (2) eosin G in phosphate buffer pH 6.6, and (3) thiazine dye in phosphate buffer pH 6.6 (Baxter Dade A.G, Düdingen, Switzerland). All experiments were performed in triplicates. As a positive control, the cells were preincubated with phorbol myristate acetate (50 ng/mL, Sigma) instead of a CD3 monoclonal
antibody. Blocking studies were performed with monoclonal antibodies directed against the common \( \beta_2 \) integrin chain (CD18): TS 1/18,\(^{10}\) against CD11a (NKI-L7),\(^{11}\) and against CD11b (Bear-1),\(^{12}\) added to the endothelial cell monolayer together with the T cells. The final antibody concentration was 10 \( \mu \)g/mL, which in previous studies was shown to result in a maximal inhibition of adhesion. The adhesion was scored blindly at 200 \( \times \) magnification by three investigators independently, in a semiquantitative way: 0 = no T cells, 1 = <50 T cells/field, 2 = 50 to 100 T cells/field, 3 = 100 to 250 T cells/field, 4 = 250 to 350 T cells/field, 5 = >350 T cells/field.

Because it is difficult to estimate the extent to which cross-linking occurs in vivo, since we do not know which part of the administered amount of CD3 monoclonal antibody actually binds to the T cells and which part remains unbound, both the whole blood incubation and adhesion studies were performed with concentrations of OKT3 or IXA ranging from 0.01 to 10 \( \mu \)g/mL. To exclude the possibility that the expression and functional activity of adhesion molecules was influenced by any antibody not directed to the CD3/T-cell receptor complex, whole blood incubations and adhesion studies were also performed with murine IgG2a monoclonal antibodies directed against either the CD4 molecule (ie, CLB-T4), or a major histocompatibility complex (MHC) class-I antigen (W6/32), instead of the CD3 monoclonal antibody. Both the CD4 and the MHC class-I monoclonal antibody were tested at concentrations of 0.1, 1, and 10 \( \mu \)g/mL.

Calculations and Statistics

**Absolute number of double-positive cells.** This was determined by the percentage of double-positive cells (calculated by means of PCLYSYS software from Becton Dickinson) multiplied by the absolute number of lymphocytes at the same time point. All values are expressed as mean \pm standard error of mean (SEM).

**Mean fluorescence intensity (MFI).** At all time points the MFI was calculated by means of PCLYSYS software. A total of 100% represents the MFI values obtained from the blood samples before the start of treatment. The values obtained several times after administration of OKT3 are represented as a percentage of the pretreatment value. All values are expressed as mean \pm SEM.

Statistical analysis. Differences within groups were tested by Wilcoxon Test for Matched pairs. A probability (\( P \)) value < .05 was considered to indicate a significant difference.

**RESULTS**

**In Vivo Studies**

**Effect of OKT3 administration on peripheral T lymphocytes.** As seen in Fig 1, treatment with 5 mg OKT3 caused within 3 minutes in all of the 10 patients studied a sharp decline in the number of CD3\(^+\) peripheral blood lymphocytes (from 1.24 \( \pm \) 0.15 \( \times \) 10\(^9\)/L to 0.07 \( \pm \) 0.01 \( \times \) 10\(^9\)/L, \( P < .05 \)). From 3 minutes on, the number of goat antimouse F(ab\(^{\prime}\))\(^2\) positive cells decreased in parallel, while the number of CD3\(^+\) cells remained very low during the whole study period. Likewise, the absolute number of CD2\(^+\) peripheral blood lymphocytes showed within 3 minutes a rapid decrease from 1.40 \( \pm \) 0.16 \( \times \) 10\(^9\)/L to 0.54 \( \pm \) 0.06 \( \times \) 10\(^9\)/L (\( P < .05 \)) (Table 1). Afterwards, the decrease in CD2\(^+\) lymphocytes was more gradual, until a lowest value of 0.08 \( \pm \) 0.01 \( \times \) 10\(^9\)/L was reached, persisting for at least 4.5 hours. Only a partial recovery to 0.29 \( \pm \) 0.11 \( \times \) 10\(^9\)/L was observed after 24 hours (ie, immediately before administration of the second OKT3 dose).

Effect of OKT3 administration on the expression of adhesion molecules on peripheral blood lymphocytes. Data obtained before and at 3, 10, 60, 120, and 240 hours after administration of the first dose of OKT3 are summarized in Table 1. The MFI of CD62L on the remaining CD3 positive cells decreased within 120 minutes to 52.41 \( \pm \) 12.86% of pretreatment value (\( P < .05 \)). The MFI of CD11a on the remaining CD2 positive cells showed a 2.5-fold increase after 3 minutes and remained high for the whole study period, while the MFI of CD11a on the remaining CD3 positive cells increased much less. The MFI of NK1-L16 on the remaining CD3 positive cells showed hardly any change (Table 1). Before OKT3 administration, 22.86 \( \pm \) 4.39% of the CD2 positive and 18.55 \( \pm \) 3.71% of the CD3 positive lymphocytes were CD11b positive. However, from 10 minutes after OKT3 administration, almost all the remaining CD2\(^+\)CD3\(^-\) cells were CD11b positive, and these CD11b\(^+\)CD2\(^+\) cells followed the total number of CD2 positive lymphocytes. These remaining CD11b\(^+\)CD2\(^+\)CD3\(^-\) appeared to be also CD16 positive (data not shown), a phenotype characteristic for natural killer cells. The MFI of CD11b on the remaining CD2 positive cells showed a 1.8-fold increase within 3 minutes following administration of OKT3 and remained high for the whole study period. The MFI of CD11b on the remaining CD3 positive cells also showed a 1.9-fold increase within 10 minutes and remained elevated for at least 4.5 hours. No changes were observed in the percentage of CD49d positive cells or in the MFI of CD49d on the remaining CD2 positive and CD3 positive population after administration of OKT3 (data not shown).

In the 10 patients from the control group (treated with 500 mg methylprednisolone only), the absolute numbers of CD2\(^+\) peripheral blood lymphocytes initially increased from
cells and neither did the expression of the adhesion molecules CD62L, CD11a/CD18, and CD49d/CD29 on CD2 positive and CD3 positive lymphocytes change (data not shown). In contrast, the number of CD3 positive lymphocytes expressing the activation epitope NKI-L16 on the CD11a/CD18 complex, showed a twofold increase within 3 minutes after addition of 1 \( \mu \)g/mL OKT3 (Fig 2A), followed by a gradual decrease. Addition of 1 \( \mu \)g/mL IXA also induced a twofold increase in the number of CD3 positive lymphocytes expressing the NKI-L16 epitope on the CD11a/CD18 complex, although more slowly as compared with OKT3 (Fig 2B). No changes were detected in the absolute numbers of CD11a+CD3+ cells (Fig 2A and B), nor in MFI of NKI-L16 and CD11a, either after OKT3 or IXA (data not shown). In addition, an immediate increase in MFI of CD11b on CD3 positive lymphocytes was observed on incubation with either OKT3 or IXA, reaching a maximum at three times the preaddition value after 60 minutes incubation (Fig 3A and B). Both the increase in NKI-L16 and CD11b expression were dose dependent, reaching a maximal effect with 10 \( \mu \)g/mL for OKT3 and with 1 \( \mu \)g/mL for IXA. A total of 0.1 \( \mu \)g/mL OKT3 or IXA still induced an increased expression of both adhesion molecules, but less than that observed with 1 \( \mu \)g/mL of each monoclonal antibody, and with 0.01 \( \mu \)g/mL OKT3 or IXA, no changes in adhesion molecule expression were detectable (data not shown). No changes

### Table 1. Expression of Adhesion Molecules on CD3 Positive and CD2 Positive Cells After Administration of 5 mg OKT3

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>CD3+</th>
<th>CD62L+CD3+</th>
<th>CD11a+CD3+</th>
<th>[NKI-L16]+CD3+</th>
<th>CD11b+CD3+</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>1.40 ± 0.16</td>
<td>1.06 ± 0.19</td>
<td>1.40 ± 0.16</td>
<td>0.47 ± 0.05</td>
</tr>
<tr>
<td>3</td>
<td>1.24 ± 0.15</td>
<td>1.06 ± 0.19*</td>
<td>1.30 ± 0.22</td>
<td>0.40 ± 0.07</td>
<td>0.23 ± 0.05</td>
</tr>
<tr>
<td>10</td>
<td>0.07 ± 0.01</td>
<td>0.30 ± 0.00</td>
<td>0.10 ± 0.02</td>
<td>0.07 ± 0.01</td>
<td>0.04 ± 0.00</td>
</tr>
<tr>
<td>60</td>
<td>0.02 ± 0.01</td>
<td>0.30 ± 0.00</td>
<td>0.10 ± 0.02</td>
<td>0.07 ± 0.01</td>
<td>0.04 ± 0.00</td>
</tr>
<tr>
<td>120</td>
<td>0.01 ± 0.00</td>
<td>0.07 ± 0.00</td>
<td>0.65 ± 0.06</td>
<td>0.07 ± 0.01</td>
<td>0.04 ± 0.00</td>
</tr>
<tr>
<td>24 h</td>
<td>0.01 ± 0.00</td>
<td>0.07 ± 0.00</td>
<td>0.65 ± 0.06</td>
<td>0.07 ± 0.01</td>
<td>0.04 ± 0.00</td>
</tr>
</tbody>
</table>

All values represent mean ± SEM (n = 10).

Abbreviation: abs. no., absolute number; ND, not determined.

* \( \times 10^9/L \).

† Percent of pretreatment value.

In Vitro Studies

**Effect of OKT3 on the expression of adhesion molecules on peripheral lymphocytes.** Addition of 1 \( \mu \)g/mL OKT3 or IXA to heparinized blood from five healthy volunteers did not induce any change in the absolute numbers of CD2+ cells, CD3+ cells, and CD16+CD56+CD3- (natural killer)
occurred in the expression of NKI-L16 or CD11b on CD3 positive cells in control samples incubated with murine IgG2a monoclonal antibodies directed against either the CD4 molecule or against a MHC class-I antigen, in any concentration tested (data not shown). Irrelevant murine monoclonal antibodies of the IgG2a or IgA isotype did not induce any alterations in expression of these adhesion molecules (data not shown).

Functional adhesion studies. Functional adhesion studies were performed to test whether the increase in expression of NKI-L16 and CD11b after incubation with OKT3 or IXA affected the adhesion capacity of the T cells. Indeed, incubation of purified T cells with 1 µg/mL OKT3 or IXA in vitro caused a transient increase in adhesiveness of these cells to endothelial cell monolayers. Figure 4A demonstrates that after 3 minutes incubation of OKT3-coated T cells with goat antiserum Ig at 37°C, an adhesion score of 4.15 ± 0.22 was reached. Three minutes incubation of IXA-coated T cells with goat antiserum Ig at 37°C resulted in an adhesion score of 3.11 ± 0.26. The increased adhesiveness appeared to be transient, because after 30 or 60 minutes incubation, the adhesion scores decreased markedly (Fig 4A). Dose response curves showed that maximal adhesion of T cells to endothelium was observed with a concentration of 1 µg/mL of either OKT3 or IXA. A total of 0.1 µg/mL OKT3 or IXA could still induce T-cell adhesion to endothelium, but less than that observed with 1 µg/mL of each monoclonal antibody, while with 0.01 µg/mL OKT3 or IXA, no changes in adhesion could be detected (data not shown). As a positive con-
Blocking studies showed that coincubation of OKT3- or IXA-coated T lymphocytes with either a CD18 or a CD11a monoclonal antibody completely inhibited the increased adhesion as observed after 3 minutes incubation with goat antimouse Ig (Fig 4B). Coincubation with a CD11b monoclonal antibody resulted in partial inhibition (Fig 4B).

DISCUSSION

In line with previous reports, the present study demonstrates that administration of the murine IgG2a antibody OKT3 causes an immediate peripheral T lymphocytopenia. Studies as to the causative mechanism(s) of this initial rapid disappearance of CD2+CD3+ lymphocytes are hampered by this very same phenomenon, because cells are no longer available for functional studies ex vivo. The decrease in the expression of CD66L on CD3+ T lymphocytes may reflect activation, leading to shedding of CD66L from the cell surface. Alternatively, it could reflect a preferential disappearance from the peripheral blood compartment of T cells with high CD66L expression. The latter possibility is supported by our observation that in the experiments in vitro, no change in expression of CD66L on T cells occurred. The increase in the percentage of CD11b+ and CD16+ cells within the remaining CD2−CD3+ population suggests that the observed increase in expression (MFI) of CD11a and CD11b and the decrease in MFI of CD62L on the remaining CD2−CD3+ lymphocytes results from a selective enrichment in the peripheral blood compartment of natural killer cells, which are indeed known to have a higher CD11a and CD11b expression, unpublished results), as well as a lower CD66L expression as compared with T lymphocytes. The increase in MFI of CD11b on the remaining CD3+ lymphocytes might reflect an actual upregulation of this adhesion molecule, as was already described by Muto et al.

These investigators showed that 30 minutes stimulation of T cells with 10 ng/mL phorbol myristate acetate at 37°C resulted in a twofold increase in the MFI of CD11b on T cells, as well as a twofold increase in the percentage of T cells expressing CD11b. This increased CD11b expression on T cells could not be blocked by cycloheximide, suggesting the presence of cytoplasmic (granule) stores of CD11b in the T cells. Phorbol myristate acetate is a direct activator of protein kinase C. Activation of T cells via the T-cell receptor/CD3 complex can also stimulate the inositol phospholipid mechanism, thereby giving rise to activation of protein kinase C, which, in turn, may result in a rapid increase in CD11b expression.

To discriminate between the two possibilities of either redistribution of cells between peripheral blood and other lymphoid compartments or real upregulation of adhesion molecules, the expression of these adhesion molecules was also measured on peripheral blood lymphocytes in vitro at various time points after addition of OKT3, as well as IXA, to whole blood of healthy control individuals. If IgA antibodies can indeed cause an upregulation of adhesion molecules, this could explain the immediate peripheral blood lymphocytopenia that occurs after in vivo administration of a CD3 antibody-coated cells with human autologous serum (data not shown).

Blocking studies showed that coincubation of OKT3- or IXA-coated T lymphocytes with either a CD18 or a CD11a monoclonal antibody completely inhibited the increased adhesion as observed after 3 minutes incubation with goat antimouse Ig (Fig 4B). Coincubation with a CD11b monoclonal antibody resulted in partial inhibition (Fig 4B).

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trol, T lymphocytes were incubated with 50 ng/mL phorbol myristate acetate. This resulted in an adhesion score of 4.67 ± 0.37. As a negative control, T cells were incubated with murine IgG2a monoclonal antibodies directed against either CD4 or a MHC class-I antigen. No changes in adhesion score were observed in any of the concentrations tested (not shown). Irrelevant murine monoclonal antibodies of the IgG2a or IgA isotype did not induce any alterations in adhesion

The theoretical possibility that circulating human antimouse antibodies, which could be present in serum and can bind to the CD3 monoclonal antibody on one site and with Fe receptors on the other site, was excluded because the presence of human antimouse antibodies could not be detected by FACS analysis after incubating CD3 monoclonal

antibody-coated cells with human autologous serum (data not shown).

Blocking studies showed that coincubation of OKT3- or IXA-coated T lymphocytes with either a CD18 or a CD11a monoclonal antibody completely inhibited the increased adhesion as observed after 3 minutes incubation with goat antimouse Ig (Fig 4B). Coincubation with a CD11b monoclonal antibody resulted in partial inhibition (Fig 4B).
monoclonal antibody of the IgA class,\(^9\) despite the absence of Fc receptors for murine IgA in humans and the inability of murine IgA to activate human complement.

In contrast to the results obtained from the studies with OKT3 in vivo, no change in expression of CD11a was observed in vitro, although an immediate, marked increase was observed in the absolute numbers of NKI-L16 positive T cells after addition of either OKT3 or IXA. Because the monoclonal antibody NKI-L16 recognizes a Ca\(^{2+}\)-dependent activation epitope on the CD11a/CD18 complex, its expression can be used as a parameter for an activation state of this adhesion molecule. However, it is known that expression of the NKI-L16 epitope by itself is not sufficient for cell-cell adhesion. Only after specific triggering of a lymphocyte by CD3 monoclonal antibodies through the T-cell receptor/CD3 complex, the NKI-L16 epitope becomes capable of high-affinity ligand binding.\(^{12,13}\) Thus, the increased expression of this activation epitope, as demonstrated in the present study, may quite well play a role in the mechanism underlying the rapid decrease of CD11a\(^{[NKI-L16]}\) CD2\(^+\) CD3\(^+\) cells in the peripheral blood compartment after OKT3 administration, as these cells will strongly adhere to ICAM-1 and ICAM-2 on vascular endothelium. In contrast, CD11a\(^{[NKI-L16]}\) CD2\(^+\) CD3\(^-\) cells would remain in the circulation, not being activated via the T-cell receptor/CD3 complex, which is a prerequisite for induction of the high-avidity state of the CD11a/CD18 molecule. The observed increase in expression of CD11b on CD3-positive lymphocytes after the addition of either OKT3 or IXA in vitro is compatible with our findings in vivo after administration of 5 mg OKT3 and may play an additional role in adhesion of T lymphocytes to ICAM-1 on vascular endothelium.

Indeed, incubation of T lymphocytes with either OKT3 or IXA appeared to induce an increased, transient adhesiveness of these cells to endothelial cell monolayers within 3 minutes. Because this increase was completely inhibited in the presence of CD11a or CD18 monoclonal antibodies, and partly in the presence of a CD11b monoclonal antibody, the induced activation status of the CD11a/CD18 molecule appears to be an absolute prerequisite for the immediate adhesion of T cells to vascular endothelium as induced by CD3 monoclonal antibodies. However, part of the increased adhesion is also dependent on increased expression of CD11b/CD18.

The CD2\(^+\)CD3\(^-\) lymphocytes that remained detectable in the peripheral blood compartment appeared to be CD11b\(^+\) and CD16\(^+\), a phenotype characteristic for natural killer cells. The ongoing decrease in absolute number of such natural killer cells between 60 and 120 minutes after the first OKT3 administration might be caused by a release of interleukin-2, which is known to occur within 2 hours after the first dose of 5 mg OKT3.\(^{22,24}\) Interleukin-2 is able to induce an immediate selective disappearance of natural killer cells from the peripheral blood,\(^{35}\) as can be explained by an increased adhesion of these cells to endothelium as was shown by in vitro studies.\(^{23-27}\) In contrast, administration of methylprednisolone did not induce a disappearance, but a transient increase of CD11b\(^+\)CD2\(^+\) cells, followed by a gradual, mild decrease, as described previously.\(^{28,29}\)

The persistence of lymphocytopenia after administration of OKT3 (or other anti-CD3 monoclonal antibodies of the IgG2a isotype) may be caused by at least two possibly cooperating mechanisms: It may result from opsonization of T lymphocytes by OKT3 and complement, followed by sequestration of such sensitized lymphocytes in the mononuclear phagocyte system. Alternatively, it may result from upregulation of VCAM-1 and ICAM-1 on the endothelium, as induced by cytokines like tumor necrosis factor \(\alpha\) and interferon \(\gamma\), which are known to be released following OKT3 administration\(^{22,24}\) and which may lead to increased adhesiveness of the endothelium for its counterstructures CD49d/CD29 and CD11a/CD18 or CD11b/CD18, respectively, expressed on peripheral blood lymphocytes.\(^{30,33}\) Evidence for upregulation of VCAM-1 by CD3 monoclonal antibodies was provided by Bergese et al\(^{32}\) after administration of the hamster monoclonal antibody 145-2C11 in mice. Because the murine IgA isotype switch variant CD3 monoclonal antibody does not bind to human Fc receptors, does not induce complement activation, and hardly results in release of cytokines, administration in vivo of IgA anti-CD3 antibody does not lead to persistent lymphocytopenia.\(^{34,35}\)

In conclusion, we provide evidence that on administration of CD3 monoclonal antibodies in vivo, the immediate peripheral blood lymphocytopenia is, at least partly, explained by changes in the expression of adhesion molecules on the surface of T lymphocytes, leading to increased adhesiveness of these cells to vascular endothelium.

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REFERENCES

1. Norman DJ: Rationale for OKT3 monoclonal antibody treatment in transplant patients. Transplant Proc 25:1, 1993 (suppl 1)
T-CELL ADHESION MOLECULES AFTER CD3 MoAb


