Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 2946–2950, May 1987
Immunology

Induction of synthesis of the cytolytic C9 (ninth component of complement)-related protein in human peripheral mononuclear cells by monoclonal antibody OKT3 or interleukin 2: Correlation with cytotoxicity and lymphocyte phenotype

(natural killer cell/cytotoxic T lymphocyte/lymphokine-activated killer cell/mechanism of cellular cytotoxicity)

DALE E. MARTIN, LEORA S. ZALMAN, GUNDRAM JUNG, AND HANS J. MÜLLER-EBERHARD

Division of Molecular Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037

Contributed by Hans J. Müller-Eberhard, January 15, 1987

ABSTRACT **Synthesis of the cytolytic C9-related protein (C9RP) was induced by activation of resting human peripheral T lymphocytes with the anti-CD3 antibody OKT3 or interleukin 2. Comparison of cellular cytotoxicity and C9RP content at various times during activation yielded a coefficient of correlation $r = 0.92$. During OKT3 stimulation of peripheral mononuclear cells, maximal C9RP content and cytotoxicity were observed by day 2 or 3, with subsequent decline to baseline values by day 5, whereas during interleukin 2 stimulation, both parameters reached the maximal level at days 3–5. After fluorescence-activated cell sorting, C9RP and cytotoxicity were quantitated in $CD4^+$, $CD8^+$, and Leu-19^+ subsets. In OKT3-activated $CD8^+$ cells, C9RP increased to $\approx 3 \times 10^6$ molecules per cell, with a corresponding increase in lysis of human melanoma cells mediated by anti-CD3–anti-melanoma monoclonal antibody conjugates. Interleukin 2-stimulated $CD8^+$ cells showed similar increases, but cytotoxicity was conjugate-independent. Activated $CD4^+$ cells showed minimal increase in C9RP content. Leu-19^+ cells, which exhibit natural killer cell activity, had a high C9RP content ($\approx 2.5 \times 10^6$ molecules per cell) before stimulation.**

Human large granular lymphocytes (LGLs), which include the natural killer (NK) cell population, have been shown in this laboratory to contain a M_r 70,000 protein that is immunochemically related to C9, the ninth component of complement (1–5). The C9-related protein (C9RP) was isolated from the cytoplasmic granules of these cells by anti-C9 affinity chromatography and shown to form, in the presence of Ca^{2+}, transmembrane channels in liposomes and to lyse K562 erythroleukemia cells. Certain monoclonal antibodies (mAbs) capable of reacting with C9RP inhibited the killing of K562 cells by LGLs, indicating that the protein was directly operative in the cytotoxic mechanism of LGLs. These cells were largely Leu-7- and Leu-11 (CD16)-positive and proliferated in the presence of interleukin 2 (IL-2).

Identification of C9RP as the cytolytic effector protein of human LGLs raised the question as to whether human cytotoxic T lymphocytes (CTLs) also utilize this protein for the execution of target membrane attack. Human peripheral CTLs are largely resting cells lacking cytotoxic activity. They require activation, which is physiologically provided by the binding of antigen to the T-cell antigen receptor. Antigen-independent activation of all $CD3^+$ peripheral CTLs may be achieved with mAb to the CD3 structure of the receptor, such as OKT3 (6). As shown in this laboratory, OKT3 stimulation of peripheral blood mononuclear cells (PBMCs) results in the generation of potent killer cells within 2–3 days (7) and their major histocompatibility complex (MHC)-unrestricted cytotoxicity may be demonstrated by use of OKT3–anti-target cell mAb conjugates. When PBMCs were depleted of $CD16^+$ cells, representing the majority of the LGLs and NK cells, and subsequently activated with OKT3, the resulting population consisted primarily of $CD4^+$ and $CD8^+$ large lymphoblasts. The cytolytic, pore-forming protein of these cells was identical with C9RP of LGLs with respect to molecular weight, reactivity with anti-C9RP, and specific cytolytic activity toward human melanoma cells and K562 cells (8). It was concluded, therefore, that both populations, the OKT3-activated $CD3^+$ CTLs and the IL-2-stimulated LGLs, employed C9RP as their pore-forming, cytolytic protein.

Having established that C9RP is the cytolytic protein of both types of human killer lymphocytes, the opportunity arose to study the induction of C9RP synthesis upon lymphocyte activation and to correlate C9RP content with expression of cellular cytotoxicity both in total PBMCs and in subpopulations.

MATERIALS AND METHODS

Human Peripheral Blood Mononuclear Cells. PBMCs were isolated from heparin-treated normal blood by Ficoll-Hypaque (Pharmacia) density gradient centrifugation and were cultured in RPMI 1640 medium (M. A. Bioproducts, Walkersville, MD) supplemented with 2 mM L-glutamine, 50 μg of gentamicin per ml, and 10% heat-inactivated fetal bovine serum. Certain cultures were stimulated with human recombinant IL-2 (Amgen Biologicals, Thousand Oaks, CA) at 100 units/ml and others with OKT3 at 100 ng/ml. The antibody was purified from hybridomas (American Type Culture Collection).

Phenotypic Analysis and Cell Sorting. PBMCs were phenotyped by using phycoerythrin- or fluorescein isothiocyanate (FITC)-conjugated mAbs: OKT4 (anti-CD4) and OKT8 (anti-CD8) (Ortho Diagnostics); anti-Leu-11a (anti-CD16) and anti-Leu-19 (NKH-1) (Becton Dickinson); and IL-2R1 (anti-IL-2 receptor) (Coulter). OKT3 (anti-CD3) was used as a primary antibody at 10 μg/ml for 30 min on ice, followed by FITC-conjugated goat anti-mouse $F(ab')_2$ (Tago, Burlingame, CA) at a 1:30 final dilution. One- or two-color fluorescence measurements were performed on a FACS IV (Becton Dickinson) flow cytometer. Fluorochrome-conjugated, isotype-matched controls were used to determine the percentage of positive cells. Cell sorting of PBMCs was performed on a FACSTAR (Becton Dickinson) cell sorter.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: C9, ninth component of complement; C9RP, C9-related protein; CTL, cytotoxic T lymphocyte; FITC, fluorescein isothiocyanate; IL-2, interleukin 2; LGL, large granular lymphocyte; mAb, monoclonal antibody; NK cell, natural killer cell; PBMC, peripheral blood mononuclear cell.

Measurement of RNA and DNA Synthesis. RNA staining was performed as described (9). Cells were incubated with 4 μM pyronin Y (Sigma) for 45 min at 37°C prior to analysis on the FACS IV. DNA synthesis was measured by 5-bromo-2'-deoxyuridine incorporation (10). Cells were incubated with 10 μM bromodeoxyuridine (Sigma) for 30 min at 37°C. After three washes in phosphate-buffered saline (PBS), cells were fixed in 70% ethanol for 30 min on ice, followed by DNA denaturation with 4 M HCl for 30 min. After neutralization with 0.1 M $Na_2B_4O_7$, cells were incubated with FITC-conjugated anti-bromodeoxyuridine mAb (Becton Dickinson) for 30 min on ice, washed, and resuspended in PBS containing propidium iodide (5 μg/ml; Sigma) for analysis on the FACS.

Conjugation of Monoclonal Antibodies. mAb 9.2.27, which is directed against a human melanoma-associated chondroitin sulfate proteoglycan (11), was conjugated with OKT3 by use of *N*-succinimidyl 3-(2-pyridyldithio)propionate (Pierce) as described (12).

Target Cells. M21 cells, a human melanoma cell line originally provided by D. L. Morton (University of California at Los Angeles), were maintained in RPMI 1640 medium supplemented as described above.

Cytotoxicity Assays. M21 cells (1.5×10^6 per ml) were labeled with ^{51}Cr (200 μCi/ml; 1 Ci = 37 GBq) for 90 min at 37°C and washed twice. Aliquots of 1.5×10^4 cells were added to 96-well microtiter plates and allowed to adhere overnight at 37°C. OKT3–9.2.27 mAb conjugate was added to some wells at 3 μg/ml for 30 min at 37°C. After washing, effector cells were added and the mixtures were incubated at 37°C for 4 hr. After centrifugation, supernatants were removed for radioactivity measurement, and cytotoxicity was calculated using a standard formula (7). Each experimental point in the killing assays represents the average of duplicate determinations. The effector/target cell ratio in all assays was 10:1.

Purification of C9RP. C9RP was isolated from IL-2-stimulated human LGLs (1, 5) and from CD16-depleted, OKT3-activated human PBMCs (8) by immunoaffinity chromatography on a Sepharose-anti-human C9RP column.

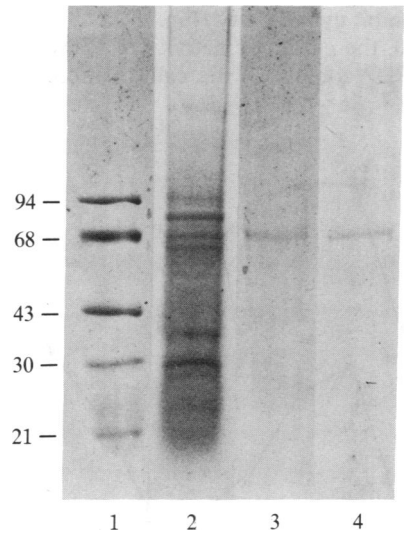

FIG. 1. $NaDodSO_4$/PAGE of C9RP isolated from OKT3-activated $CD3^+$ human CTLs (lane 3) and from IL-2-stimulated human LGLs (lane 4). Also shown (lane 2) is the pattern of a lysate (60 μg of protein) of PBMCs activated for 3 days with OKT3 (100 ng/ml). The amount of C9RP was 5 μg, the gel gradient was 1.7–17%, and staining was by Coomassie brilliant blue. Molecular weight standards are shown in lane 1 ($M_r \times 10^{-3}$ at left).

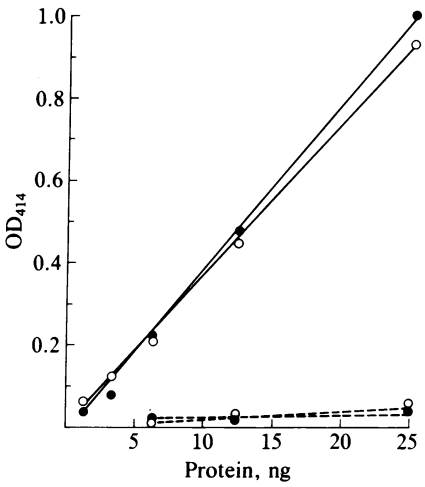

FIG. 2. Standard curves, obtained with C9RP from CTLs (○) and from LGLs (●) by ELISA, used for quantitation of C9RP in cell lysates. Specificity is shown by inhibition resulting from preincubation of anti-C9RP with an excess of isolated C9RP (broken lines).

ELISA. Lymphocytes were washed in RPMI 1640, suspended in 0.1 M carbonate buffer (pH 9) containing 2 mM EGTA, and disintegrated by freezing and thawing six times, and the cell lysates were obtained after centrifugation. Total protein was quantitated by the Bio-Rad assay (Bio-Rad), using bovine gamma-globulin as standard. Microtiter plates were coated with cell lysates at 4°C for 16 hr and blocked with 0.2% (wt/vol) nonfat milk (Carnation) in PBS. Protein A-purified $F(ab')_2$ fragment of rabbit anti-human C9RP was used at various dilutions, followed by peroxidase-conjugated $F(ab')_2$ fragment of goat anti-rabbit IgG (Tago) at 1:5000 dilution. 2,2'-Azinobis(3-ethylbenzthiazolinesulfonic acid) (Boehringer Mannheim) was used as substrate. Standard curves were derived from C9RP purified from human LGLs or activated CTLs (5, 8).

RESULTS

Quantitation of C9RP in Cell Lysates. Samples of $5–15 \times 10^6$ cells were disrupted by freezing and thawing, and the C9RP content in the cell lysates was quantitated by ELISA using as standards C9RP isolated from IL-2-stimulated LGLs and from OKT3-activated $CD3^+$ CTLs (Fig. 1). Standard

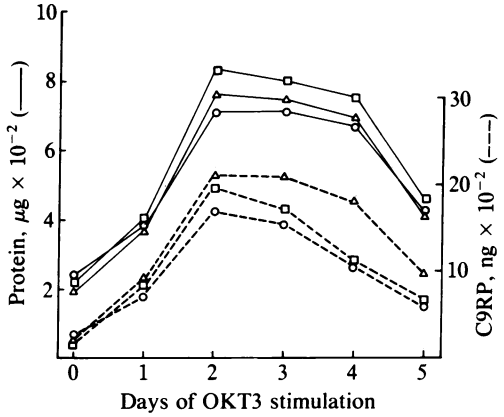

FIG. 3. Induction of C9RP synthesis in PBMCs by OKT3. PBMCs from three donors phenotypically characterized (Table 1) were stimulated with OKT3 (100 ng/ml) for 0–5 days. The cell lysates were analyzed for C9RP (ng per 10^7 cells) by ELISA and for total protein (μg per 10^7 cells) by a Coomassie blue G-250 assay (Bio-Rad protein assay).

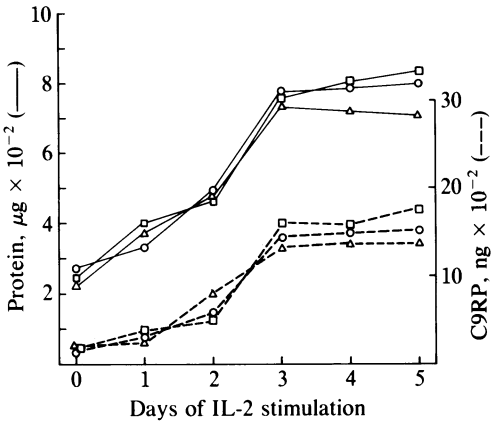

FIG. 4. Induction of C9RP synthesis in PBMCs by IL-2. PBMCs from three donors phenotypically characterized (Table 1) were stimulated for 0–5 days with IL-2 (100 units/ml). Protein (μg) and C9RP (ng) per 10^7 cells were quantitated as described for Fig. 3.

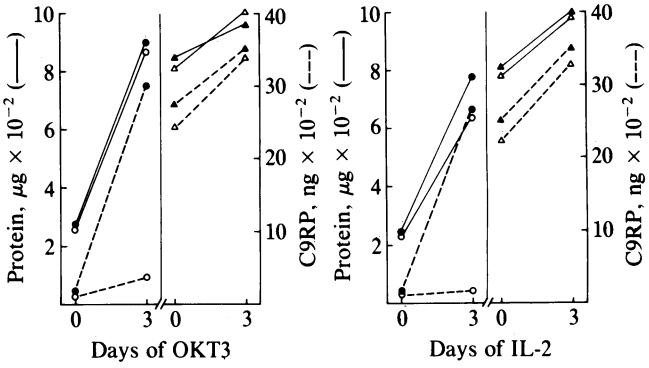

FIG. 5. Induction of C9RP synthesis in subsets of PBMCs activated with OKT3 or IL-2 prior to sorting. Reanalysis of the sorted populations indicated >98% purity. C9RP content of the $CD8^+$ cells (●) increased 15- to 20-fold, and that of $CD4^+$ cells (○), only 2-fold. Leu-19^+ cells (▲, Leu-19^+,11^+; △, Leu-19^+,11^-) exhibited a high C9RP content already before stimulation. Protein and C9RP values are expressed as in Figs. 3 and 4.

curves obtained with $F(ab')_2$ of rabbit anti-human C9RP are shown in Fig. 2. Specificity of the reaction was demonstrated by inhibition of the antibody by excess antigen. The second antibody in the ELISA was also used in the form of $F(ab')_2$ to avoid binding to Fc receptors possibly present in the cell lysates.

Induction of C9RP Synthesis in PBMCs by OKT3 or IL-2. PBMCs cultured with OKT3 or IL-2 for various times were harvested for quantitation of total protein and C9RP content. Fig. 3 depicts the results of OKT3 stimulation of PBMCs from three normal donors for 0–5 days. The maximum of C9RP and total protein content was reached by day 2, with a marked decline at days 4 and 5. C9RP represented ≈1% of total protein. Fig. 4 shows the results of IL-2 stimulation of PBMCs.

C9RP Synthesis in PBMC Subsets. PBMCs stimulated for 0 or 3 days with OKT3 or IL-2 were phenotyped with mAbs (Table 1) and sorted to >98% purity. Both $CD4^+$ and $CD8^+$ cells responded to OKT3 with a marked increase in protein synthesis (Fig. 5). This is consistent with the light microscopic observation that nearly all treated PBMCs were large lymphoblasts (data not shown). C9RP content rose sharply in the $CD8^+$ population, to ≈3×10^6 molecules per cell, and only slightly in the $CD4^+$ cells. The Leu-19^+,11^+ and Leu-19^+,11^- subpopulations, appearing as LGLs by light microscopy (data not shown), had a high C9RP content already before stimulation (≈2.5×10^6 molecules per cell) and experienced only a moderate increase in C9RP after 3 days of stimulation. The same subpopulations sorted after 3 days of IL-2 stimulation displayed a very similar pattern of C9RP and total protein content (Fig. 5).

RNA and DNA Synthesis in Stimulated PBMCs. Pyronin Y staining followed by flow cytometry showed that the RNA content of the activated PBMCs rose markedly during the first 2–3 days (Fig. 6). When the kinetic analysis of C9RP synthesis (Figs. 3 and 4) is compared with that of RNA synthesis, a close correlation is apparent. In contrast, DNA synthesis, reflecting the proliferative response to the stimuli, lagged considerably.

Table 1. Phenotypic analysis of PBMCs stimulated by OKT3 or IL-2

PBMCs	% positive cells					
	CD3	CD4	CD8	IL-2R	CD16	Leu-19
Unstimulated	78 ± 6	45 ± 9	29 ± 7	4 ± 1	11 ± 4	14 ± 5
OKT3; day 3	83 ± 7	47 ± 8	34 ± 6	67 ± 7	7 ± 4	20 ± 6
IL-2; day 3	79 ± 7	44 ± 9	30 ± 5	22 ± 6	8 ± 5	19 ± 8

Values are means ± SEM for PBMCs from three donors. IL-2R, IL-2 receptor.

Correlation Between C9RP Content and Cellular Cytotoxicity. Cytotoxicity was measured using M21 human melanoma cells as targets. Both PBMC populations, stimulated with OKT3 or IL-2, acquired maximal cytotoxic activity toward M21 cells and maximal C9RP concentration during the first 2–3 days (Figs. 7 and 8). To assess the contribution of CD3-dependent cytotoxicity to the total cytotoxic capacity of the PBMCs, the 9.2.27–OKT3 mAb conjugate was employed. A 30–50% increase in cell killing was observed with OKT3-activated PBMCs and a 15–20% increase with IL-2-stimulated PBMCs. In the case of OKT3 activation, intracellular C9RP concentration and cytotoxicity declined in parallel after day 3, whereas a plateau was established for both parameters in IL-2-stimulated PBMCs.

The cytotoxic activity of the $CD8^+$ subset of OKT3-activated PBMCs was entirely dependent on the mAb conjugate. However, the $CD8^+$ cells from IL-2-activated PBMCs showed no such conjugate dependence. This lack of dependence on the CD3 structure was verified by the inability of OKT3 to inhibit cytotoxicity (Fig. 9).

In Fig. 10, the cytotoxic activity of PBMCs is compared with the C9RP content of the cells. Different cell samples

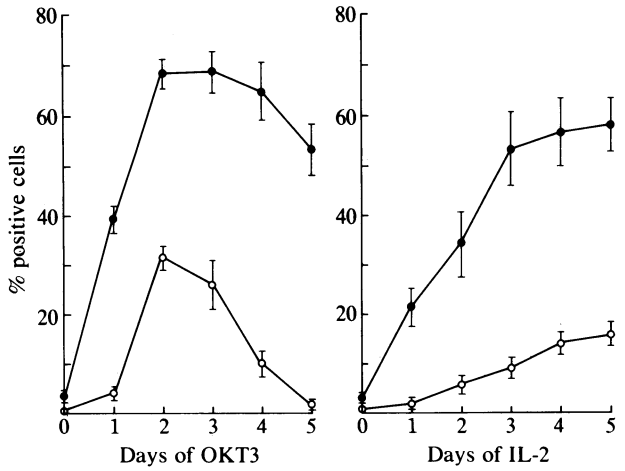

FIG. 6. RNA (●) and DNA (○) synthesis in OKT3- or IL-2-stimulated PBMCs. The cells were stained with pyronin Y (RNA) or with anti-bromodeoxyuridine mAb (DNA), and the percentage of positively stained cells was determined by fluorescence cytometric analysis. Each point represents the mean ± SEM of 3 experiments.

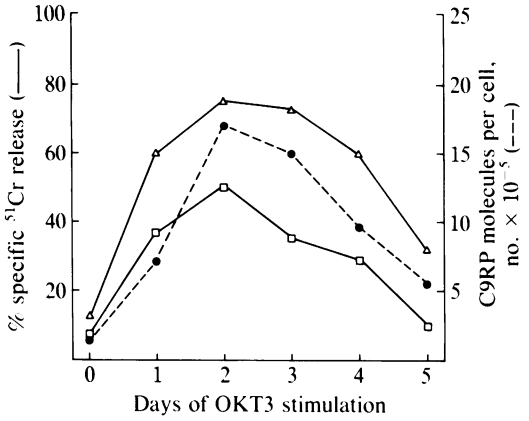

FIG. 7. Cellular cytotoxicity (solid lines) and C9RP content (broken line) of PBMCs stimulated by OKT3 for 0–5 days. ^{51}Cr-labeled M21 human melanoma cells were incubated for 4 hr with PBMCs at an effector/target ratio of 10:1 with (△) or without (□) 9.2.27–OKT3 mAb conjugate.

from two donors were treated with either stimulus for various periods before analysis. By linear regression analysis, the coefficient of correlation was 0.92.

DISCUSSION

The insights gained into cellular cytotoxicity through these results are as follows. (*i*) C9RP synthesis is induced in CTLs upon activation of these cells. Resting CTLs, which exhibit no cytotoxic activity, contain little or no C9RP. (*ii*) The extent of cytotoxic activity of killer lymphocytes bears a direct quantitative relation to the amount of C9RP contained in these cells. Since isolated C9RP is cytolytic (5, 8), these observations indicate that the cytotoxic potential of killer lymphocytes depends, at least in part, on C9RP. (*iii*) Unlike CTLs, freshly isolated NK cells have a high content of C9RP, consistent with their known cytotoxic activity. (*iv*) $CD8^+$ cells of IL-2-stimulated PBMCs acquired a high content of C9RP during activation and expressed major histocompatibility complex (MHC)-unrestricted cytotoxicity that was also independent of involvement of their CD3 structure.

Both methods of stimulation of PBMCs resulted in polyclonal activation. Although total protein synthesis increased in $CD4^+$ and $CD8^+$ cells to the same extent, very little C9RP was synthesized in $CD4^+$ cells. There was a >20-fold increase (from $\approx 10^5$ to $\approx 3 \times 10^6$ molecules per cell) in C9RP content of $CD8^+$ cells, but only a 2-fold increase in $CD4^+$

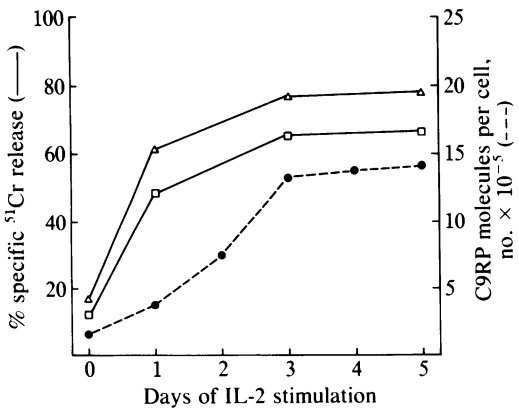

FIG. 8. Cellular cytotoxicity (solid lines) and C9RP content (broken line) of PBMCs stimulated with IL-2 for 0–5 days. Cytotoxicity was assayed as for Fig. 7, in the presence (△) or absence (□) of 9.2.27–OKT3 conjugate.

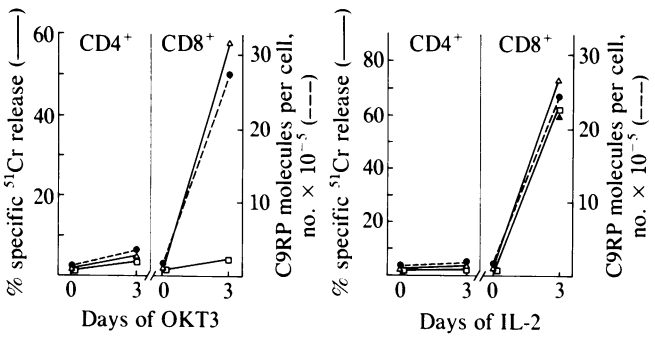

FIG. 9. $CD4^+$ or $CD8^+$ subpopulations sorted from PBMCs stimulated with OKT3 or IL-2 for 0 or 3 days: Correlation between cellular cytotoxicity and C9RP content. Cytotoxicity was assayed with mAb conjugate (△—△), without conjugate (□—□), or with conjugate plus OKT3 (75 μg/ml) (▲).

cells. The increase in $CD4^+$ cells is, however, biologically significant, since they do acquire a low but definite degree of cytotoxic potential (13, 14).

Whereas the cytotoxicity of OKT3-activated $CD8^+$ cells was entirely dependent upon anti-target–anti-CD3 mAb conjugates, that of IL-2-stimulated $CD8^+$ cells was almost entirely conjugate-independent. Although these two sets of $CD8^+$ cells had similar cytotoxic potentials, the killing mechanism of the OKT3-activated cells required the engagement of the CD3 structure, whereas killing by IL-2-activated cells did not. The molecular mechanisms underlying these functional differences are not understood.

In unstimulated PBMCs, the Leu-19^+ (NKH-1) subpopulation (≈15% of total PBMCs) contained high levels of C9RP ($\approx 2.5 \times 10^6$ molecules per cell). This phenotype has been shown to encompass the NK activity in freshly isolated PBMCs (15) and to have LGL morphology. The majority of these Leu-19^+ cells are also Leu-11^+ ($CD16^+$)—i.e., are endowed with Fcγ receptors—but ≈25% are $CD3^+$ (Leu-19^+ $CD3^+$ $CD16^-$), and their cytotoxicity can be blocked by OKT3 (16). Both Leu 19^+ $CD16^+$ and Leu 19^+ $CD16^-$ subsets responded to IL-2 or OKT3 stimulation with only modest increases in their originally high C9RP content (<50%).

Induction of C9RP synthesis in relation to cytotoxicity could only be demonstrated with resting, noncytotoxic lymphocytes that were capable of acquiring cytotoxic potential upon activation. In almost all related previous studies, cytotoxic cell lines or cloned cells were used, which already contain considerable amounts of cytolytic protein, so that

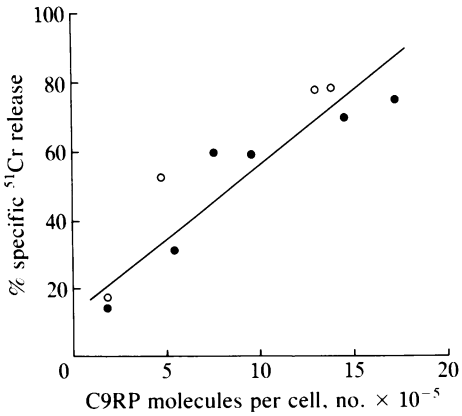

FIG. 10. Quantitative relation between C9RP content and cellular cytotoxicity of different PBMC samples activated either by OKT3 (●) or IL-2 (○). The cells were derived from two different donors and stimulated for various times prior to analysis. The line was obtained by linear regression analysis (correlation coefficient $r = 0.92$).

induction of synthesis of perforin (17, 18), cytolysin (19), or pore-forming protein (20), which are analogous terms for C9RP, was not observed. One report (20) that confirmed the original isolation of C9RP from human NK cells (1, 5) indicated failure to detect C9RP in $CD3^+$ cells after activation of PBMCs with 40–50 units of IL-2 per ml for 3 days. In the present study, IL-2 was used at 100 units/ml. In another report (21), lymphokine-activated killer cells were generated by incubation of murine spleen cells with IL-2 at 100 units/ml, and cytolysin activity, which was initially undetectable, appeared after 2 days of culture.

Recently, the expression of CTL-specific gene products was investigated at the cDNA (22, 23) and protein (24, 25) level. By both approaches, a serine protease was identified that was expressed with kinetics correlating with acquisition of cytolytic capacity. The possible mechanistic relation of this enzyme to C9RP induction remains to be elucidated.

This is publication no. 4702-IMM from the Department of Immunology, Research Institute of Scripps Clinic. This investigation was supported by National Institutes of Health Grants AI17354, CA27489, and HL16411. D.E.M. is the recipient of a National Institutes of Health Physician-Scientist Award administered through the University of California, San Diego. L.S.Z. is supported by National Institutes of Health Training Grant HL07195.

1. Zalman, L. S., Brothers, M. A. & Müller-Eberhard, H. J. (1985) *Biosci. Rep.* **5**, 1093–1100.
2. Zalman, L. S., Brothers, M. A., Chiu, F. J. & Müller-Eberhard, H. J. (1985) *Complement* **2**, 90 (abstr.).
3. Müller-Eberhard, H. J. (1986) *Annu. Rev. Immunol.* **4**, 503–528.
4. Zalman, L. S., Brothers, M. A., Chiu, F. J. & Müller-Eberhard, H. J. (1986) *Sixth International Congress on Immunology* (National Research Council Canada, Ottawa, Canada), p. 195 (abstr.).
5. Zalman, L. S., Brothers, M. A., Chiu, F. J. & Müller-Eberhard, H. J. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 5262–5266.
6. Weiss, A., Imboden, J., Hardy, K., Manger, B., Terhorst, C. & Stobo, J. (1986) *Annu. Rev. Immunol.* **4**, 593–619.
7. Jung, G., Honsik, C. J., Reisfeld, R. A. & Müller-Eberhard, H. J. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4479–4483.
8. Zalman, L. S., Martin, D. E., Jung, G. & Müller-Eberhard, H. J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2426–2429.
9. Shapiro, H. M. (1981) *Cytometry* **2**, 143–150.
10. Dolbeare, F., Gratzner, H., Pallavicini, M. G. & Gray, J. W. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 5573–5577.
11. Bumol, T. F. & Reisfeld, R. A. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1245–1249.
12. Carlsson, J., Drevin, H. & Axen, R. (1978) *Biochem. J.* **173**, 723–737.
13. Weber, W. E. J., Buurman, W. A., Vandermeeren, M. M. P. P. & Raus, J. C. M. (1985) *J. Immunol.* **135**, 2337–2342.
14. Jung, G., Martin, D. E. & Müller-Eberhard, H. J. (1987) *J. Exp. Med.*, in press.
15. Phillips, J. H. & Lanier, L. I. (1986) *J. Exp. Med.* **164**, 814–825.
16. Schmidt, R. E., Murray, C., Daley, J. F., Schlossman, S. F. & Ritz, J. (1986) *J. Exp. Med.* **164**, 351–356.
17. Masson, D. & Tschopp, J. (1985) *J. Biol. Chem.* **260**, 9069–9072.
18. Young, J. D. E., Podack, E. R. & Cohn, Z. A. (1986) *J. Exp. Med.* **164**, 144–155.
19. Henkart, P. A. (1985) *Annu. Rev. Immunol.* **3**, 31–58.
20. Liu, C. C., Perussia, B., Cohn, Z. A. & Young, J. D. E. (1986) *J. Exp. Med.* **164**, 2061–2076.
21. Henkart, P. A., Yue, C. C., Yang, J. & Rosenberg, S. A. (1986) *J. Immunol.* **137**, 2611–2617.
22. Gershenfeld, H. K. & Weissman, I. L. (1986) *Science* **232**, 854–858.
23. Lobe, C. G., Finlay, B. B., Paranchych, W., Paetkau, V. H. & Bleackley, R. C. (1986) *Science* **232**, 858–861.
24. Pasternack, M. S., Verret, C. R., Liu, M. A. & Eisen, H. N. (1986) *Nature (London)* **322**, 740–743.
25. Young, J. D. E., Leong, L. G., Liu, C. C., Damiano, A., Wall, D. A. & Cohn, Z. A. (1986) *Cell* **47**, 183–194.