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Effect of Oxidized Cholesterol Derivatives on Lymphokine-Stimulated Macrophage Differentiation and Primary Allogenic Mixed Culture of Human Lymphocytes

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Products of cholesterol oxidation, 25-hydroxycholesterol and 7-ketocholesterol, in a dose of 1 μg/ml considerably suppress proliferative response in a primary one-way mixed culture of human lymphocytes and have no effect on spontaneous proliferation. Preliminary 18-h separate culturing of the stimulator and responders with these lipids shows that inhibiting effect of these lipids on cell proliferation is associated with their influence on both stimulator and, to a greater extent, responders. Both lipids in the same dose inhibit accumulation of HLA-DR-positive macrophages in a 4-day culture of peripheral blood adherent cells stimulated with lymphokine-containing supernatant.

Key Words: *oxidized cholesterol; macrophages; HLA-DR expression; mixed lymphocyte culture*

Cell activation and immune recognition via HLA-DR overexpression in atherosclerotic lesions are associated with lymphokine production by $CD4^+$ T cells infiltrating these lesions [5,7,8]. On the other hand, the role of oxidized cholesterol (CH) seems to be an important aspect in the interplay between disturbed cholesterol metabolism and macrophage function [1,6]. 25-Hydroxycholesterol (25-hCH) and 7-ketocholesterol (7-kCH) exhibit antiproliferative activity and activation of macrophages is accompanied by enhanced production of these metabolites [1,2]. A question arises: whether 25-hCH and 7-kCH affect macrophage differentiation and efficiency of immune recognition? The present study explores the *in vitro* effect of these compounds on a primary allogenic mixed culture of human lymphocytes and expression of HLA-DR during macrophage differentiation from peripheral monocytes upon stimulation with lymphokines.

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MATERIALS AND METHODS

Test lipids were isolated from mixed product of CH oxidation by high-performance thin-layer chromatography [2], dissolved in freshly distilled ethanol, and added to cell cultures so that the final ethanol concentration was 0.1%. Among other controls, the addition of CH ethanol solution of an equivalent concentration was used.

Mononuclear cells were isolated from Na_2EDTA-stabilized human blood by centrifugation in a Ficoll-Verografin gradient (d=1.078 g/cm^3). Blood was ob-

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tained from healthy donors: 8 men and 6 women aged 25-45 years. Primary one-way mixed culturing of human lymphocytes (MLC) was carried out in the presence of 20% pooled AB(IV) serum as described previously [3]. In other experimental series MLC was carried with twice washed lymphocytes after their preincubation with lipids (1 μg/ml, 18 h). The reaction was assessed by ^{3}H-thymidine incorporation on day 6 of MLC.

Test lipids in a concentration of 1 μg/ml were also added to a culture of adherent peripheral blood cells. Supernatant of two-way MLC was used as a lymphokine source as described for cultured mouse lymphocytes [4]. We used this approach for cultured human mononuclear cells: 5×10^6 cells in 200 μl medium [3] were incubated for 1.5 h at 37°C in a flat-bottom plate in the presence of 10% fetal calf serum; nonadherent cells were removed, while adherent cells in 100 μl medium were cultured for 4 days in the presence of 50 μl supernatant of 48-h two-way MLC stored at -70°C as a source of lymphokines. After incubation, HLA-DR-positive macrophages were counted under a light microscope (Trypan Blue exclusion test) after complement-dependent lysis using monoclonal antibodies to nonpolymorphous HLA-DR (N. N. Blokhin Oncology Research Center, Russian Academy of Medical Sciences).

The data were processed statistically using the Wilcoxon paired test and Wilcoxon—Mann—Whitney test for independent samples.

RESULTS

Analysis of proliferative activity in 6 MLC showed that neither spontaneous, nor stimulated proliferation depended significantly on the dose of test lipids (5, 1, and 0.1 μg/ml), therefore in further experiments lipids were added in a dose of 1 μg/ml. Lipids (1 μg/ml) and solvent (0.1% ethanol) had no effect on lymphocyte proliferation in a mixed culture. On day 6 of MLC the level of spontaneous proliferation in the presence and absence of ethanol was 509±105 and 468±96 cpm, respectively ($p>0.05$). The level of spontaneous proliferation in the presence of 25-hCH, 7-kCH, and CH did not differ from the control and constituted 570±70, 514±58, and 427±109 cpm, respectively. This allowed us to use stimulation index (stimulated to spontaneous proliferation ratio). Ethanol had no significant effect on stimulated proliferation (stimulation index in the presence of ethanol and in the control was 4.38±1.2 and 4.55±1.04, respectively, $p>0.05$) and on cultured adhesive cells.

25-hCH and 7-kCH markedly suppressed (by 79.6 and 75%) lymphocyte proliferation in MLC; stimulation index was 0.93±0.71 and 1.14±0.21, respectively ($p<0.01$). Cholesterol exerted a weaker statistically significant effect: stimulation index was 3.73±0.85, lymphocyte proliferation was suppressed by 19.1% in comparison with the control (Fig. 1, *a*).

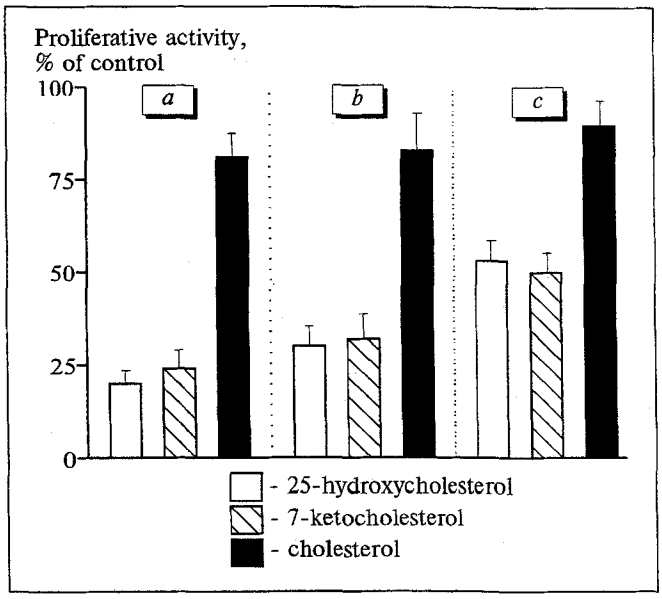

Fig. 1. Effect of oxidized cholesterol derivatives on proliferative response in a primary one-way mixed culture of human lymphocytes. *a*) in the presence of lipids; *b*) preincubation of responders with lipids; *c*) preincubation of stimulators with lipids. Data of 8 healthy donors.

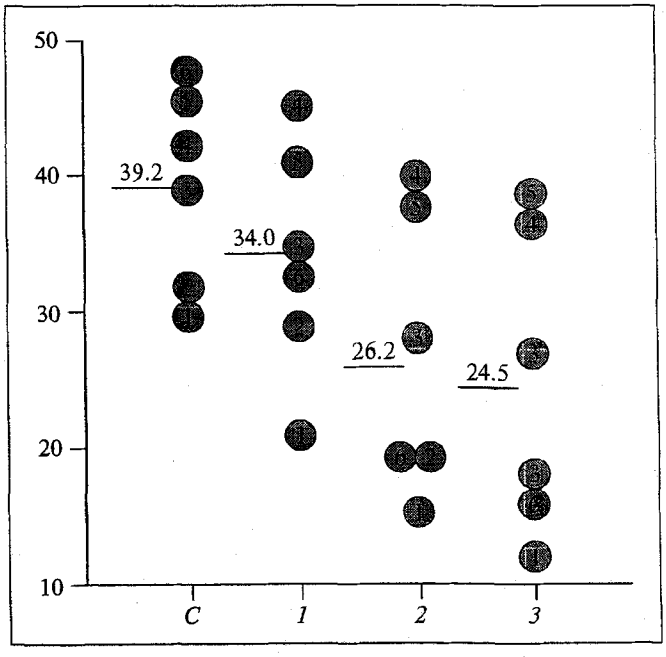

Fig. 2. Effect of oxidized cholesterol derivatives on the number of HLA-DR-positive macrophages in lymphokine-stimulates cultures. Ordinate: % of HLA-DR-positive macrophages. The cells were cultured in the presence of lymphokines solely (*C*) or together with cholesterol (*1*), 7-25-hydroxycholesterol (*2*), and 7-ketocholesterol (*3*). Points *1-6*: individual values of 6 donors; figures on lines are the mean numbers of DR-positive cells.

The fact that 25-hCH and 7-kCH dramatically suppressed stimulated lymphocyte proliferation in MLC and had no effect on spontaneous proliferation suggests that antiproliferative effect of these lipids is realized at the level of the activation of signal induction.

Preliminary 18-h separate incubation of responders and stimulators showed that inhibition of lymphocyte proliferation is associated with the effect of test lipids on both stimulators (Fig. 1, *c*; $p<0.01$) and, to a greater extent, responders (Fig. 1, *b*; $p<0.01$). Cholesterol again exerted a moderate effect on cell proliferation.

The effect of CH on accumulation of HLA-DR-positive macrophages in the presence of lymphokines (Fig. 2, *3*) was insignificant for the given 6 donors ($p>0.05$); a tendency toward a decrease in this parameter was noted. On the other hand, oxidized CH metabolites significantly decreased the number of DR-positive macrophages ($p<0.01$, Fig. 2, *1*, *2*). Despite the roughness of this estimation, it can be noted that 25-hCH and 7-kCH reduced the number of DR-positive macrophages by 20% (7-60%) and 38.5% (12-65%), respectively, in comparison with control cultures (cultures with lymphokines but without exogenous lipids; Fig. 2, *C*). Differentiation of peripheral monocytes to DR-positive macrophages occurred in control cultures in the absence of lymphokines (data not shown), but the number of these cells did not differ from that in cultures with lymphokines and oxidized CH metabolites, i.e., 25-hCH and 7-kCH abolish the stimulating effect of lymphokines.

Thus, 25-hCH and 7-kCH inhibit macrophage differentiation and induction of antigen-specific proliferation by affecting both antigen-presenting and antigen-recognizing cells.

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