15-Deoxysperguallin (a New Guanidine-like Drug) Blocks T Lymphocyte Proliferation

W. Falk, K. Ulrichs, and W. Müller-Ruchholtz

15-DEOXYSPERGUALIN (15-DS), a guanidine-like derivative of sperguallin, is presently being investigated as a new immunosuppressive drug in several transplantation models, and shows a remarkable effect, comparable to that of cyclosporine A (CsA) concerning survival of allografts. However, unlike CsA, 15-DS is believed to exert its effects preferentially on macrophages, though its precise mode(s) of action remain(s) widely unknown to this date.

MATERIAL AND METHODS

Indirect immunofluorescence

Splenic macrophages of AS ($RT1^l$) rats were incubated in vitro with varying concentrations of 15-DS (0.01–500 μg/mL) for one to four days; major histocompatibility complex (MHC) class II antigen expression was tested with the OX6 (anti I-A) antibody in indirect immunofluorescence. Positive cells were read as a percentage of the total cell count and compared to untreated controls.

Mixed Lymphocyte Culture

One hundred thousand LEW ($RT1^l$) splenic lymphocytes as responder cells and 100,000 mitomycin C-treated DA ($RT1^{av1}$) splenic lymphocytes as stimulator cells, and cocultivation for 5 days were used; ^{3}H-thymidine uptake was measured as counts per minute (cpm). Experimental group I: permanent incubation with 0.01–2.0 mg/mL 15-DS for 8–112 hours was carried out. Group II: preincubation of 10^7 stimulator cells with 0.2–2.0 mg/mL 15-DS for three to 16 hours was carried out. Group III: preincubation of 10^7 responder cells with 0.2–2.0 mg/mL 15-DS for three to 16 hours was carried out.

Phytohemagglutinin Stimulation

One hundred thousand LEW,DA or Balb/c splenic lymphocytes or peripheral human blood lymphocytes were stimulated with 30 μg/mL PHA, and coincubated with 0.05–2.0 mg/mL 15-DS for 56 hours. ^{3}H-thymidine uptake was measured as cpm.

RESULTS

15-DS, tested in concentrations ranging from 0.01 to 500 μg/mL, decreases the cell surface expression of major histocompatibility complex (MHC) class II (I-A) antigens of AS splenic macrophages by about 32% ($P < .01$), as can be observed with the help of indirect immunofluoresence. The effect is dose-dependent but time-independent with regard to the duration of the drug incubation.

In the mixed lymphocyte culture (MLC) experimental group I with permanent presence of 15-DS during the 5-day culture period, a dose-dependent inhibition of the allogeneic immune response is observed (Fig 1). A significant inhibition can still be achieved when 15-DS is added as late as 16 hours before cell harvesting. This effect is dose dependent. In experimental group II, preincubation of the stimulator cells with 15-DS before mitomycin C blockage has no inhibitory effect when such cells are cocultured with untreated responder cells. Variations of the 15-DS dose and incubation times do not alter this result. In experimental group III, preincubation of the responder cells with 15-DS prior to coculture with untreated stimulator cells results in a significant dose- and time-dependent inhibition of the immune response. The effect is significant for concentrations ranging from 0.5 to 2.0 mg/mL and six to 16 hours incubation time. Shorter periods of incubation are ineffective.

Phytohemagglutinin (PHA) stimulation of either rat or mouse splenic lymphocytes or peripheral human blood lymphocytes can also

From the Department of Immunology, Medical School of the University, Brunswikerstr. 2-6, D-2300 Kiel, FRG.

Address reprint requests to W. Falk, Department of Immunology, Medical School of the University, Brunswikerstr. 2-6, D-2300 Kiel, FRG.

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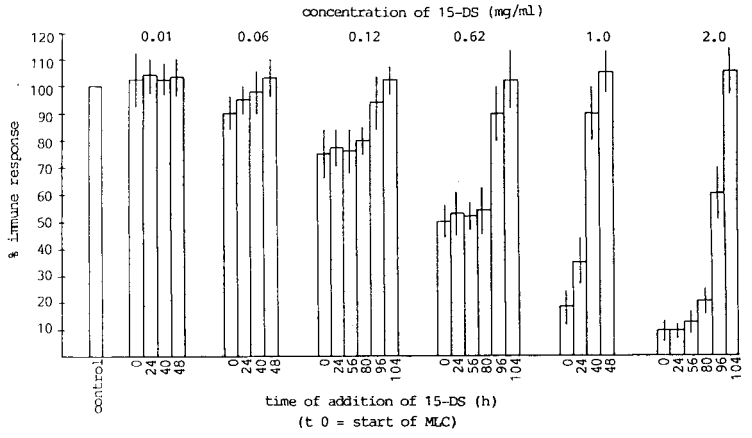

Fig 1. Mixed lymphocyte culture (permanent incubation of 15-DS): 15-DS still exerts a dose-dependent inhibitory effect when added as late as 16 hours before cell harvesting (n = 7).

be inhibited by 15-DS in a dose-dependent fashion.

DISCUSSION

The proposition that 15-DS is effective against macrophages is supported by indirect immunofluorescence examination indicating a reduction of macrophage MHC class II antigen expression. Thus, 15-DS may act as an inhibitor of antigen presentation. However, inhibition of the MLC by 15-DS as late as 16 hours before cell harvesting appears to hint at modes of action independent of these macrophages, since antigen presentation is known to be the initiating event of cell interactions. 15-DS may well exert an effect on T cell reactivity, as suggested by the preincubation experiments. Additional attempts, eg, interleukin-1 and interleukin-2 assays, are presently under way to gain a clearer view of the possible different modes of action of 15-DS.