plasm would not be bound to calcium[18]. It is not clear to what extent the immunostaining procedure interferes with the binding of calcium to calmodulin or with the subcellular distribution of calcium. Binding of radiolabeled calmodulin to the cytoplasmic surface of neurosecretory granules has, however, been shown to occur both in the presence and in the absence of calcium[8].
The subcellular, granular distribution of calmodulin in the cells investigated in the present study is compatible with the involvement of calmodulin in exocytosis.

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Selective killing of smooth muscle cells in culture by the ricin A-chain conjugated with monoclonal antibodies to a cell surface antigen via a dextran bridge

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Summary. Monoclonal antibodies to a surface antigen of the modulated smooth muscle cells originally isolated from the rat aorta media were conjugated with ricin A-chain via an oxidized dextran bridge. The interaction of cultured cells with the conjugates obtained and with control substances was monitored following incorporation of ^{14}C-leucine radioactivity. It was found that ^{14}C-leucine incorporation was suppressed by 80–90% at a conjugate concentration of 10^{-6}–10^{-7} M. Antigen-negative cells (line IAR; rat hepatocytes) were insensitive to the conjugate at any concentration used. Control use of purified ricin A-chain, native or oxidized dextran, specific and nonspecific IgG did not affect normal ^{14}C-leucine incorporation. The data obtained may be useful for designing targeted drug transport systems and for selective screening of modulated smooth cells in vascular pathology models in vivo.
Key words. Smooth muscle cells; monoclonal antibodies; ricin A-chain; drug transport-system; dextran bridge.

It is known at present that in different disease states (including widely-distributed ones such as atherosclerosis and hypertension) smooth muscle cells participate in the pathological process; some of them leave the resting state and enter the cell cycle, which ends in either true cell replication or endoreplication[1,2]. It was shown in cell culture models that only cells whose phenotype is modified into the 'synthetic' one can enter a proliferative phase[3,4]. The discovery of markers specific for the modulated cells is very important for the localization of these cells in a normal vessel, for the investigation of their role in vascular pathology and for the construction of systems for targeted transport of biologically active compounds to these cells. Using model cellular cultures with analogous modifications of smooth muscle cells monoclonal antibodies were obtained to surface antigens of modulated cells, which were originally isolated from the media of rat aorta. One of the monoclonal antibodies obtained, termed L_1, interacts specifically only with a surface antigen of cultured smooth muscle cells and does not bind with cells of other types[5,6].
At present antibodies to cell surface antigens are widely used for immunotoxin synthesis and selective action on the antigen-bearing cells[7].

The aim of the present work was to study the possibility of the use of monoclonal antibodies L_1 as vectors for targeted transport of model compounds to the modulated smooth muscle cells. It is known that direct binding of a toxin or another biologically-active substance to an antibody sometimes noticeably affects antibody specific properties or does not permit the binding of a sufficient quantity of active molecules to each immunoglobulin molecule. To solve the problem drugs (for example, antibiotics) can be coupled to antibodies via a polymeric bridge, particularly via an activated dextran macromolecule[8,9].
In our case dextran activated by periodate oxidation was used as a polymeric bridge and the A-chain of the plant toxin ricin was used as a model active substance. In principle, instead of one or a few ricin A-chains a larger number of low-molecular-weight molecules can be bound with a dextran bridge, capable of targeted action on smooth muscle cells entering into proliferation in different pathological states.
The activity of the conjugates obtained, i.e. the ability of L_1-antibodies to be modified, without loss of activity, with a reactive polymer capable of binding biologically active compounds, was observed by following the selective killing of antigen-positive smooth muscle cells in vitro.

Materials and methods. Cell cultures. Long-term cultured smooth muscle cells from the media of rat aorta (SMM), in the 10–15th passage, were isolated and cultured as described earlier[10]. Rat hepatocytes, line IAR-2, were used as antigen-negative cells.

Monoclonal antibodies were obtained after the immunization of BALB/c mice with smooth muscle cells and the fusion of murine splenocytes with the cells of myeloma P3-8Ag-653[5]. Antibodies were precipitated from the ascitic fluid with ammonium sulfate.

Ricin A-chain. Ricin from *Ricinus communis* seeds was obtained by ammonium sulfate fractionation, affinity chromatography on Biogel A–0.5 m (Bio-Rad) and gel-filtration on Sephadex G-100 according to Nicolson and Blunstein[11]. The ricin molecule consists of a receptor B-chain (R_B, 31.4 kD) and a catalytic A-chain (R_A, 30.6 kD) which are bound together by S–S bonding. After S–S bond reduction R_A was isolated by ion-exchange chromatography on DEAE- and CM-cellulose[12]. The traces of R_B were separated on Bio-gel A-0.5 m. The purity of R_A was estimated by gel-electrophoresis in polyacrylamide in the presence of SDS[13] and by isoelectric focusing on Multiphor. Inhibiting activity was determined in a cell-free protein-synthesizing system from rabbit reticulocytes[14].

R_A conjugates. Dextran (Pharmacia) with a mol. wt of 35,000–50,000 was activated with KIO_4 as in[15]. The degree of oxidation was circa 10%. For binding with dialdehydedextran (DAD) 2×10^{-5} M R_A and 4×10^{-5} M DAD were incubated in phosphate buffer at 4 °C for 18 h with mixing. Bound R_A was separated on a K-16 column (Pharmacia) with Sephacryl S-300 (Pharmacia). The binding yield was 50%. Then the solution of modified R_A (10^{-5} M) in phosphate buffer was supplemented with 0.5×10^{-5} M solution of L_1 or nonspecific IgG (MOPC) in the same buffer and the mixture was incubated at 4 °C for 18 h with mixing. Molar ratio R_A:DAD:IgG = 2:8:1. The triple conjugate was separated by gel-chromatography on Sephacryl S-300. IgG binding yield was 10–15% (see fig. 1, A, B). The conjugate was additionally purified by dialysis against distilled water for 2 h and against 0.01 M phosphate buffer, pH 7.0 for 4 h and then lyophilized.

The inhibition of protein synthesis in target cells was monitored by ^{14}C-leucine incorporation. SMM cells were placed in 24-well plates (Linbro) with a density of about 10^5/ml in leucine-free medium MEM (Flow) with the addition of 10% FCS (Flow) at 37 °C, 95% air, 5% CO_2. After 10–12 h of incubation with the samples under investigation the system was supplemented with ^{14}C-leucine (338 Ci/mmol, Amersham) up to a final concentration of 0.6 μCi/ml. 2.5–3 h later cells were aspirated on GF/C filters (Whatman). Then the filters were dried and sample radioactivity was measured in a liquid scintillation counter LKB Rackbeta 1215. The incorporation of ^{14}C-leucine into non-treated cells was taken as 100% (circa 30,000 cpm). Nonspecific radioactivity adsorption was circa 1000 cpm. The action of R_A-DAD-L_1 was compared with the action of R_A-DAD-IgG (MOPC); pure R_A, native ricin, pure L_1 and pure IgG (MOPC). Rat hepatocytes IAR-2 line were used as control antigen-negative cells.

Results and discussion. The monoclonal antibody used, L_1, specifically binds with a surface antigen of cultured SMM cells[5] and does not interact with rat fibroblasts, thymocytes, red blood cells, skeletal muscle cells or hepatocytes. The expression of L_1 antigen in the primary culture correlates with the binding of the L_1 monoclonal antibody in the logarithmic growth phase of SMM[6]. This means that L_1 probably binds with the surface of modulated SMM cells which begin to proliferate. Thus, L_1 can be used as a vector in an immunotoxin conjugate for selective action on modified SMM cells.

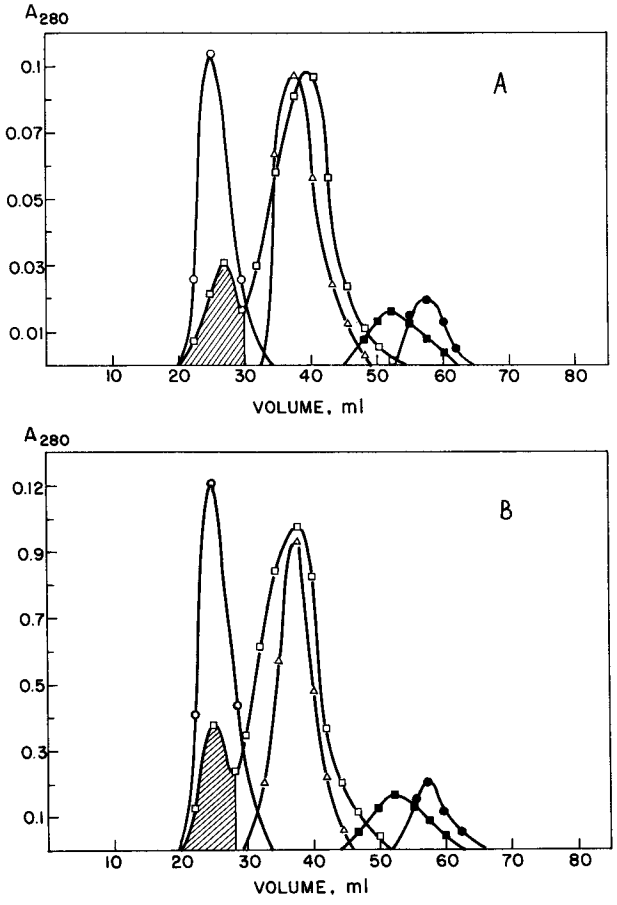

Figure 1. *A* Gel-chromatography of R_A-DAD-L_1 (□), pure L_1 (△), R_A-DAD (■), and R_A (●). *B* Gel-chromatography of R_A-DAD-IgG (MOPC) (□), pure IgG (MOPC) (△), R_A-DAD (■) and R_A (●). A, B – Blue dextran (○). Sephacryl S-300, K-16 (Pharmacia) column (see Methods for details).

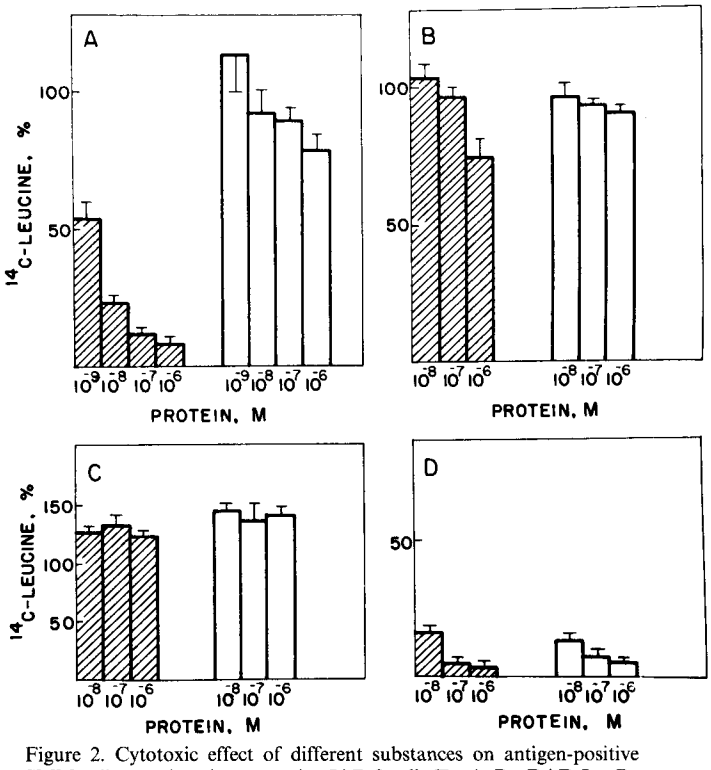

Figure 2. Cytotoxic effect of different substances on antigen-positive SMM cells ▨ and antigen-negative IAR-2 cells □ : A–R_A-DAD-L_1; B–R_A-DAD-IgG (MOPC); C–pure L_1; D–ricin.

Under the conditions suggested, triple conjugates of the R_A-DAD-IgG type can easily be obtained with a satisfactory yield (fig. 1). The R_A-DAD-L_1 conjugate preserves its ability to bind with the surface of SMM cells (indirect fluorescence measurements, data not shown), whereas the intermediate compounds R_A-DAD and R_A-DAD-IgG (MOPS) do not bind to SMM cells (see figs 2 and 3).

It was found that at a concentration of 10^{-6}–10^{-7} M the conjugate R_A-DAD-L_1 suppressed ^{14}C-leucine incorporation into SMM cells by 90% and practically did not influence ^{14}C-leucine incorporation into control IAR-2 cells. Pure native ricin at the same concentrations suppresses ^{14}C-leucine incorporation in both cell types by 90–95%. A nonspecific conjugate of the R_A-DAD-IgG (MOPC) type did not decrease protein synthesis either in SMM or in IAR-2 cells (figs 2, 3). Control use of pure R_A, native or oxidized dextran, specific and nonspecific IgG only slightly affect normal ^{14}C-leucine incorporation (protein synthesis) in both cell types; the maximal decrease observed was about 20% in the case of R_A.

Thus, only the R_A-DAD-L_1 conjugate specifically binds SMM cells and suppresses protein synthesis, causing cell death.

The data obtained show that L_1 antibodies can be used as vectors for targeted transport of different substances to the modulated smooth muscle cells. This is particularly helpful for the selective screening of proliferating smooth muscle cells in vascular pathology models in rats[16,17].

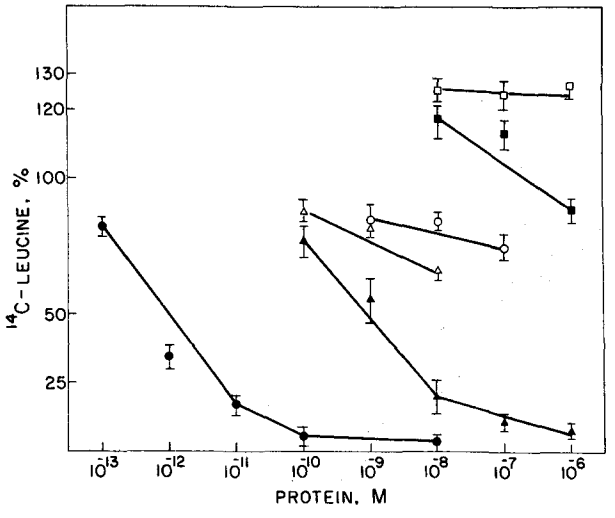

Figure 3. Cytotoxic effect on SMM cells of different concentrations of ricin (●), R_A (○), R_A-DAD-L_1 (▲), R_A-DAD-IgG (MOPC) (■), R_A-DAD (△), pure L_1 (□).

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Contrasting effects of RNA and protein synthesis blocking on natural and lectin-dependent cell-mediated cytotoxicity against adherent HEp-2 cells

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Summary. In this study, earlier observations[2,9] concerning the independence of both natural (NCMC) and lectin-dependent cell-mediated cytotoxicity (LDCC) from DNA synthesis have been confirmed. In addition, blocking of RNA synthesis by actinomycin D and of protein synthesis, reversibly by puromycin (PM) and irreversibly by emetine (EM) had different effects on NCMC and LDCC against ^{3}H-thymidine-prelabeled HEp-2 target cells. Similarly to the Con A-induced proliferation of lymphocytes, LDCC activity was also inhibited by blocking of RNA and protein synthesis. NCMC to HEp-2 target cells was not affected by blocking of RNA synthesis, while both PM and EM strongly enhanced NCMC activity.

Key words. Natural cell-mediated cytotoxicity; lectin-dependent cell-mediated cytotoxicity; RNA synthesis; protein synthesis.

The molecular mechanism of cell-mediated cytotoxicity has recently prompted extensive investigaitons[1,4]. In all types of in vitro cytotoxic reactions, such as specific T cell-mediated and nonspecific lectin-dependent (LDCC), oxidation-dependent (ODCC) as well as natural killer (NK) cell-mediated cytotoxicity, after binding of the effector lymphocyte to the target cell, the 'lethal blow' is thought to be delivered by the interaction of preformed cell surface structures[1,4] on the effector and target cells. Berke has suggested that one cytotoxic T cell receptor has two functions: binding to and lysis of the target cell[1], whereas Bonavida et al. reported that binding and lysis can be separately blocked by different specific antibodies[4]. We have earlier demonstrated profoundly depressed levels of LDCC in patients with systemic lupus erythematosus[10] and carcinoma of the uterine cervix[11], using a sensitive and reproducible LDCC assay, by detachment from the monolayer of ^{3}H-thymidine-prelabeled HEp-2 adherent target cells with concanavalin A (Con A). These observations have supported the putative role of endogeneous lectins and/or lectin-like receptors[8] in cytotoxicity changes observed in primary and secondary diseases of the immune system. In this cytotoxicity system the major effector cells are T lymphocytes. Both $OKT4^+$ and $OKT8^+$ T cell subsets and LDCC