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Selective Adsorption of Human CD4⁺ T Cells

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Abstract: The pathogenesis of most autoimmune diseases directly involves CD4⁺ helper T cells. To remove CD4⁺ T cells selectively from the circulation, we designed a new column in which an anti-CD4 monoclonal antibody was immobilized on the activated substance. Nearly 90% of CD4⁺ T cells were selectively adsorbed from whole blood with a single passage through the column in vitro, resulting in depletion of the antigen-specific T cell responses. We conclude that this new column would be potentially useful for treatment of T cell-mediated autoimmune diseases.
Key Words: Cytapheresis, CD4 helper T cell, Immunotherapy.

Antigen (Ag)-specific CD4⁺ T cells play a central role in the pathogenesis of most of autoimmune diseases (1,2). Monoclonal antibodies to the CD4 Ag have proven effective in the control of experimental autoimmune diseases (3), and uncontrolled clinical trials indicate that anti-CD4 antibody therapy is beneficial in various human autoimmune diseases (4–12). However, previous studies of murine monoclonal antibodies against various cell surface Ags in humans have revealed several major limitations of this strategy. Murine monoclonal antibodies often do not significantly deplete the function of the target cells (4,6,7,12), and it is often difficult to know how many cells can be affected after administration the antibodies. Murine antibodies also provoke a host antimouse response which may limit the effectiveness of therapy, especially with repeated treatment (5,6,12). To minimize these problems we designed a column to adsorb CD4⁺ T cells ex vivo, using anti-CD4 monoclonal antibody. Reported here are the property of the column and the effects on the human peripheral lymphocytes.

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MATERIALS AND METHODS

The column to adsorb CD4⁺ T cells consists of polystyrene non-woven-fabric on which an anti-CD4 monoclonal antibody was fixed (CD4 removal column). The monoclonal antibody, purified antihuman CD4 mouse IgG ak 2 (clone BL 4), was immobilized on the carrier using hydroxymethyliodeacetoamide, and blocked with bovine serum albumin. By not detecting particular fluorescent intensity in the filtrate when fixing antibodies conjugated with fluorescein isothiocyanate on the carrier, we confirmed that the antibodies were not released from the column after extensive washing. The column, 1 mL in volume, can treat 10–15 mL whole blood at the flow rate of 1 mL/min. A column without anti-CD4 monoclonal antibody was used for sham experiments.

We performed a flow cytometric determination of lymphocyte subsets in whole blood before and after the above treatment, using EPICS Elite (Coulter). Red blood cells were lysed with ammonium chloride (NH_4Cl), and lymphocytes were stained with anti-CD4, CD8, CD3, and CD20 antibodies conjugated to fluorescein isothiocyanate or phycoerythrin.

To assess functional activity of the lymphocytes from untreated or treated blood with the CD4- column, we measured the proliferation to mitogens and purified protein derivatives of tuberculin (PPD). First, PPD-specific CD4⁺ T cell lines were raised from the peripheral blood mononuclear cells (PBMCs), which maintained by restimulation with PPD and autologous mitomycin (MMC)-treated PBMCs and

TABLE 1. *Adsorption rate (%)† of each cell population when whole blood was treated with the control column or the CD4-column*

Cell populations	Control column mean percentage ± SD ($N = 4$)	CD4-column mean % ± SD ($N = 5$)	P-value*
RBC	3.1 ± 1.4	2.1 ± 2.2	0.45
Platelets	19.5 ± 14.1	25.3 ± 15.8	0.58
WBC	23.0 ± 7.6	18.0 ± 4.7	0.26
Granulocytes	22.6 ± 8.1	7.0 ± 3.0	0.02
Monocytes	39.4 ± 10.0	47.0 ± 9.1	0.27
Lymphocytes	31.9 ± 10.0	36.5 ± 9.8	0.51
CD3+ CD20-	29.9 ± 10.8	53.4 ± 6.1	<0.01
CD3-CD20+	41.0 ± 9.5	9.8 ± 6.1	<0.01
CD4+ CD8-	28.5 ± 10.3	88.2 ± 4.9	<0.01
CD4-CD8+	30.4 ± 11.5	10.0 ± 6.0	0.01

†, Absorption rate (%) = 100 × (cell number of pretreatment – cell number of post-treatment)/(cell number of pretreatment); *, Student's t-test or Welch's t-test was used where appropriate.

expansion with recombinant human IL-2. The autologous whole blood(10 mL) supplemented with the PPD-specific $CD4^+$ T cells(1×10^6) were treated with the column, then PBMCs were separated on a Ficoll density gradient from both untreated and treated blood, and 2×10^5 cells/well were cultured in 200 μL medium. 1×10^5 autologous MMC-treated PBMCs were added as additional Ag presenting cells because non-woven fabric filters mechanically adsorb a portion of monocytes. After 72 h of culture at 37°C in 5% CO_2, ^{3}H-thymidine was added to each well for the following 18 h, and cells were harvested on filters for scintillation counting.

RESULTS

A single pass of human whole blood through the column reduced a great number of $CD4^+$ T cells. $CD4^+$ $CD8^-$ cells decreased from 27.4 to 1.3% (mean, $N = 7$), whereas $CD8^+$ $CD4^-$ cells did not. The CD4-column selectively adsorbed significantly many more $CD4^+$ T cells than the control. Granulocytes and B cells were less adsorbed by the CD4-column. Red blood cell counts remained unchanged (Table 1).

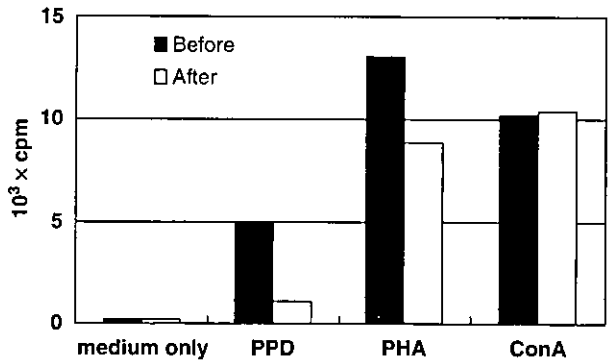

FIG. 1. Proliferative responses of peripheral blood mononuclear cells from the blood treated with the CD4-column or control column. PPD, purified protein derivatives of tuberculin; PHA, phytohemaggulutin; ConA, concanavalin A. The blood (10 mL) contains the autologous PPD-specific $CD4^+$ T cell clones(1×10^6) before treatment.

PBMCs from the untreated blood responded well to PPD (stimulation index; SI = 42.6), which was markedly suppressed after treatment with the CD4-column (SI = 7.1) but not with the control column (SI = 30.2). Lymphoproliferation to phytohemagglutinin or concanavalin A was well conserved. PBMCs which passed through the column did not proliferate spontaneously without PPD or mitogens (Fig. 1).

DISCUSSION

Although lymphocytapheresis has been used to treat autoimmune diseases with some benefit, unselective removal of lymphocytes from the peripheral blood does not appear to be a reasonable therapeutic modality (13). We demonstrated that the CD4-column efficiently adsorbed $CD4^+$ T cells directly from whole blood. In vitro, Ag-specific $CD4^+$ T cells were also adsorbed with the column, resulting in abrogation of the Ag- specific T cell responses. These results suggest that the column is potentially useful in treatment of T cell-mediated autoimmune diseases. However, like lymphocytapheresis (13), a single treatment with the column can remove target cells only from the circulation and may not be sufficient to suppress disease activity. Repeated treatment would overcome this problem. In human autoimmune diseases, clonal expansion of autoimmune T cells has been reported (14,15). If we can remove these pathogenic T cells from the circulation, further disease progression may be prevented. Along with corticosteroids for suppression of the autoimmune response and inflammation, cytapheresis with the CD4-column may benefit patients with acute

phase of autoimmune disease. This strategy would also be useful to control undesired immune responses in bone marrow transplantation.

In conclusion, the current results indicate that by selective removal of $CD4^+$ T cells, it may be possible to attenuate the $CD4^+$ T cell immune responses. The technology used in the CD4-column may be applicable to adsorb other target cells, such as lymphocytes with other surface markers or other immune cells, by selecting the appropriate antibody as a ligand. By targeting other surface molecules, such as markers for activated T cells, adhesion molecules or particular T cell receptors, we may remove the pathogenic cells more efficiently.

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