

HB7

CC chemokine receptor 6 (CCR6) is a marker for memory T cells that arrest on activated human dermal microvascular endothelium under shear stress.

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We asked whether CCR6, which is found on ~50% of memory, but not naïve, T cells is associated with the ability of memory T cells (mTC) to arrest on TNF- α activated endothelial cells under flow conditions. CCR6⁻, CCR6⁺, and total mTC were fractionated by magnetic-bead selection using CCR6 and CD45RA mAbs. TNF- α stimulation of human dermal microvascular endothelial cells (HDMEC) induced the mRNA for LARC/MIP-3 β (the chemokine ligand for CCR6) by ~200-fold as measured by real-time, quantitative RT-PCR. LARC, when coated on plastic plates, induced firm arrest of CLA⁺ mTC in a parallel plate flow chamber under physiologic shear stress (1.5 dynes/cm²). Similarly, mTC arrested on monolayers of activated HDMEC, which was inhibited 50-60% by pertussis toxin (PTX) pretreatment of mTC. Strikingly, depletion of the CCR6⁺ mTC caused >75% (n=3, p<0.01) of the loss of adhesion seen with PTX treatment of mTC. CCR6⁻ mTC had similar levels of α 2 and α 4 integrins compared with CCR6⁺ mTC and arrested on SDF-1 α , but not LARC α , coated plates. Thus, LARC, which is upregulated by activated HDMEC, induced arrest of CLA⁺ mTC *in vitro*. Depletion of CCR6⁺ mTC led to almost as much loss of adhesion on activated HDMEC as PTX-treatment of total mTC, suggesting a possible role for CCR6 and LARC in the arrest of mTC on inflamed skin endothelium.

HB9

Anti-CD11a treatment for psoriasis concurrently increases circulating T-cells and decreases plaque T-cells, consistent with inhibition of cutaneous T-cell trafficking.

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The humanized monoclonal antibody, anti-CD11a, displays several properties *in vitro*, which might lead to therapeutic benefit in psoriasis: 1) inhibition of T-cell adhesion to ICAM on endothelial cells and keratinocytes; 2) blockade of the co-stimulatory interaction of LFA-1/ICAM. In an open-label study, 11/21 (52%) and 15/24 (62%) of subjects with moderate to severe plaque psoriasis showed a \geq 50% improvement in PASI after 8 weeks of treatment with 1.0 or 2.0 mg/kg SC anti-CD11a respectively. In both groups, circulating lymphocyte count approximately doubled 2 - 7 days after the first dose of anti-CD11a. The mean proportion of circulating CD19⁺B and CD3⁺T cells remained constant. The number of accessible CD11a binding sites on circulating T cells declined \geq 95% within 48 hours of the first dose, reflecting occupancy by anti-CD11a and a 70-80% down-regulation of CD11a. All changes persisted during treatment and resolved by 6 weeks after the last dose. Skin biopsies performed after 28 days of treatment showed mean decreases of 27% and 55% in epidermal CD3⁺ T cell counts in the low and high dose groups, which declined to 58% and 63% one week after the last dose. Dermal T-cell counts declined about 60% in both groups by the end of treatment. Skin biopsies showed a decline in available T-cell CD11a, though less complete than on circulating lymphocytes. The divergence between circulating and cutaneous T-cell counts suggest that anti-CD11a may inhibit cutaneous T-cell trafficking.

HB8

Gangliosides Block Keratinocyte Binding to Fibronectin through Carbohydrate-Carbohydrate Binding to the α_5 Subunit of $\alpha_5\beta_1$.

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We have previously shown that the highly sialylated ganglioside of keratinocytes, GT1b, blocks keratinocyte interaction with fibronectin, leading to diminished adhesion and migration of a fibronectin matrix. The mechanism for this effect is unknown. The direct role of GT1b without secondary cellular components in effecting the inhibition was examined by establishing a cell-free system. Using recombinant $\alpha_5\beta_1$ and lysed keratinocyte-derived SCC12 cells, we demonstrated that GT1b decreases the binding of $\alpha_5\beta_1$ to the Arg-Gly-Asp region of fibronectin. To test the possibility of direct binding of ganglioside to integrin or its subunits, co-immunoprecipitation and three novel techniques, fluorescent bead binding assays, a slot blot analysis, and nickel-agarose binding assays were used. GT1b co-immunoprecipitates with SCC12 $\alpha_5\beta_1$, and, as shown by every assay, binds directly to SCC12 cell affinity-purified $\alpha_5\beta_1$, a mixture of insect recombinant α_5 and β_1 , and specifically to the recombinant α_5 subunit. Ganglioside GD3 is also able to bind to $\alpha_5\beta_1$, but to a lesser extent, consistent with its weaker ability to inhibit binding of keratinocytes to fibronectin. The importance of the carbohydrate moieties of integrin was shown by the inability of GT1b to bind to deglycosylated $\alpha_5\beta_1$, or inhibit its interaction with fibronectin. Competition assays with concanavalin A provided evidence that GT1b recognizes the extracellular domain α_5 integrin at a site with mannose residues. These data suggest that gangliosides serve as a co-factor in regulating $\alpha_5\beta_1$ -mediated adhesion of epithelial cells to fibronectin through direct carbohydrate-carbohydrate interactions between GT1b and $\alpha_5\beta_1$ integrin. In view of the importance of the $\alpha_5\beta_1$ /fibronectin interaction for a variety of epidermal disorders, including psoriasis, delayed wound healing, and cutaneous carcinomas, this novel interaction represents a new target for therapeutic manipulation.

HB10

Intravenous Administration of RNA Suppresses the Induction of Delayed-type Hypersensitivity (DTH) to Tumor-associated Antigens.

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Intravenous (iv) administration of protein antigens can induce relative tolerance to subsequent immunization with that antigen. We hypothesized that iv injection of RNA may also reduce cell-mediated immunity to antigens encoded by the RNA utilized. To test this hypothesis, we utilized the S1509a murine tumor system. CAF₁ mice (H-2^{d/d}) were injected with 100 μ g of total cellular RNA from the S1509a spindle cell tumor line (H-2^d). Control animals were injected iv with saline. All mice were then immunized by subcutaneous injection of disrupted S1509a cells 3 times at 6-7 d intervals. One week after the last injection, syngeneic epidermal cells enriched for Langerhans cell content (eEC) by antibody and complement-mediated deletion of Thy-1-bearing cells were pulsed with a soluble extract of the S1509a cells as a source of tumor-associated antigens (TAA). eEC were then thoroughly washed and 7.5 x 10⁵ injected into a hind footpad of each mouse. For negative controls, mice primed iv with RNA or saline alone prior to immunization were challenged with eEC not pulsed with TAA. Twenty-four hr footpad swelling was assessed as a measure of DTH. Mice given S1509a-derived RNA demonstrated a significantly smaller DTH response (p<0.001) compared to mice primed iv with saline. In a preliminary experiment, mice primed iv with unrelated RNA (derived from the NS cell line), S1509a RNA pretreated with RNase or saline pretreated with RNase prior to immunization failed to show a significantly inhibited DTH response compared to positive control mice primed iv with saline alone prior to immunization. These results demonstrate that iv administration of total cellular RNA from the S1509a tumor suppresses the induction of DTH to S1509a-derived TAA.