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Efficiency of Antibodies Directed Against Adhesion Molecules to Prolong Skin Graft Survival in Mice

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The interest to use monoclonal antibodies (MAbs) as immunosuppressive agents in allograft rejection treatment has increased considerably. Certain MAbs can block or modify critical steps in the rejection response and have the capacity to prolong graft survival, without the appearance of toxic side effects. We have studied the potential capacity of anti-adhesion molecules MAbs to inhibit the rejection of skin grafts in mice. This study focuses on the adhesion molecules that are expressed on leukocytes and mediate distinct adhesion interactions between leukocytes and between leukocytes and endothelium. LFA-1 (CD11a/CD18) is such a leukocyte-specific adhesion molecule, which binds its ligand ICAM-1 (CD54), present on leukocytes as well as endothelium. By using inhibitory MAbs directed against these adhesion receptors, in vitro studies have demonstrated that this receptor-ligand interaction (LFA-1-ICAM-1) is required for antigen-presenting cell (APC)-T-cell interaction, T-B-cell interaction, T-cell-mediated killing, as well as T-cell migration. Recently also other ligands of LFA-1 have been identified (ICAM-2 and ICAM-3), which may also contribute in these distinct LFA-1-mediated cell-cell interactions. Apart from ICAM-1 other ligands are recognized by Mac-1. Here we investigated if MAbs directed against LFA-1, Mac-1, ICAM-1, or a combination of these antibodies could enhance skin graft survival in mice.

Materials and Methods

Animals

Each group consisted of five male C57BL/6 (H-2b) (B6) recipient mice that were transplanted with two male bml (H-2DkKbml) tail skin grafts and one syngeneic skin graft on the upper part of the tail. One day before transplantation, followed by injections twice a week until the end of the experiment (maximal 80 days).

Antibodies

The rat MAbs M17.4 obtained from the American Type Culture Collection (ATCC, Rockville, Md) and H154.163, kindly provided by Dr Pierre, were directed against the murine Mac-1 α-chain; MAb YN1/1.7.4, obtained from ATCC, was directed against murine ICAM-1. The MAbs M1/70.15, obtained from ATCC, and 5C6, kindly provided by Dr M. Robinson, were directed against the murine Mac-1 α-chain.

Cell-mediated lympholysis

CML was performed by culturing B6 spleen cells for 5 days at 37°C with allogeneic bml irradiated spleen cells in Iscove's medium supplemented with 5% fetal calf serum (FCS). The cytotoxic response of the effector cells was tested by their capacity to kill 51Cr-labeled bml lipopolysaccharide blasts in a 51Cr release assay of 4 hours at 37°C. Antibodies were added during the effector phase and were used in a final concentration of 10 μg/mL.

Results

The blocking capacity of different anti-adhesion MAbs in adhesion-dependent immunologic processes, such as effector-target cell interaction, was investigated in vitro. The capacity of two anti-LFA-1 (M17.4, H154.163), two anti-Mac-1 (M1/70.15, 5C6), and one anti-ICAM-1 (YN1/1) MAb to block the cytotoxic B6 anti-bml response was determined. Both anti-LFA-1 antibodies inhibited the specific lysis of bml target cells by the B6 effector cells, anti-ICAM-1 antibodies inhibited partially, whereas anti-Mac-1 did not (Fig 1). The combination of anti-LFA-1 and ICAM-1 antibodies completely inhibited cytosis. All antibodies that blocked the cytotoxic T-lymphocyte (CTL) response were also potent inhibitors of the B6 anti-bml MLR (mixed lymphocyte reaction, not shown). Although Mac-1 antibodies did not inhibit the cytotoxic or proliferative response of the allogeneic bml-induced B6 response, these antibodies inhibited other Mac-1-dependent interactions (not shown).

The in vivo capacity of these antibodies to inhibit skin transplant rejection was determined by injecting mice intraperitoneally with 300 μg of each antibody, starting 1 day before transplantation, followed by injections twice a week until the end of experiment. This protocol was chosen because 300 μg yielded high serum levels and occupied the adhesion receptors expressed on lymphocytes.

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Antigen MAbs

*Note. Recipient mice were injected with 300 μg of each MAb starting 1 d before transplantation, followed by twice a week until the end of experiment (maximal 80 d).
where ultimately all rejected. This is in contrast to the experiments described by Isobe et al (9) who have demonstrated that tolerance was induced in mice by a combination of anti-LFA-1 and anti-ICAM-1 when transplanted with allogeneic hearts.

Although anti-Mac-1 antibodies (Mac-1 and 5C6) did not inhibit in vitro responses, when injected in vivo a significant prolongation of graft survival was observed. However, a much longer graft survival rate was observed with anti-LFA-1. This indicates that Mac-1-dependent adhesion of monocytes and granulocytes plays a less important role in the process of graft rejection than LFA-1. Because a humoral response was detected in mice injected with anti-Mac-1 antibodies, the formation of anti-rat antibodies could also attribute to the minor affects seen with anti-Mac-1 antibodies.

In conclusion we have demonstrated that antibodies directed against the adhesion molecules LFA-1, and to a lesser extent, Mac-1 lead to prolonged skin graft survival in mice.

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