trathymic islet allografts modified responsiveness to donor alloantigens, we performed several in vitro functional and phenotypic analyses of T lymphocytes. Fluorescence-activated cell sorter (FACS) analysis of lymphocyte populations revealed no differences between thymic allograft recipients and normal controls or hosts of long-standing intratesticular or renal subcapsular allografts (*14*). Similarly, mixed lymphocyte culture responses of T lymphocytes from islet allograft acceptors to donor strain stimulator cells were indistinguishable from responses to "third party" stimulators (*15*). However, limiting dilution analysis of lymph node cells revealed a significantly reduced (40 to 60%) precursor frequency of cytotoxic T lymphocytes (CTLp) to donor strain alloantigen (Lewis) in recipients of intrathymic grafts as compared to untransplanted controls (Fig. 3), suggesting that deletion or functional inactivation of class I–restricted RT1[l] reactive T lymphocytes had occurred. In the same recipients the CTLp frequency for DA alloantigens was unchanged.

Our finding that the thymus is a privileged transplant site was unexpected because, unlike privileged sites such as the brain and the hamster cheek pouch, the thymus does not lack efferent lymphatics (*13, 16*). However, thymic cortical vessels contain numerous tight junctions between endothelial cells that do not permit extravasation of particulate dyes or radiolabeled circulating cells (*17*). In addition, mature T cells rarely return to the thymus, thus making it relatively protected from immune surveillance (*18*). Though these anatomic and physiological features might account for survival of allografts implanted in the thymus, they would not explain the favorable influence on allografts subsequently implanted in extrathymic sites. We hypothesize that this influence may be attributable to the role of the thymus in the induction of self tolerance. Allogeneic thymus transplants are capable of inducing tolerance, as can intrathymic inoculation of allogeneic thymus stem cells in lethally irradiated mice that are reconstituted with syngeneic bone marrow (*19–21*). Our data indicate that allogeneic cells of neither thymic nor lymphoid origin, if present in the thymus, can induce unresponsiveness, and that this may depend on deletion, inactivation, or both.

ALS depletes the peripheral circulation of T cells and accelerates the kinetics of stem cell traffic into the thymus (*22, 23*). In rats injected once with ALS, the peripheral T cell population is transiently (14 days) depleted to 10% of normal levels as assessed by FACS analysis (*14*). Subsequent reconstitution of the peripheral T lymphocyte pool in these animals presumably requires that prothymocytes migrate to and mature in the thymus. In our experiments, prothymocytes were forced to mature in a thymus containing cells expressing foreign MHC antigens.

These studies demonstrate that pancreatic islet allografts transplanted into the thymus survive indefinitely and are capable of inducing donor-specific unresponsiveness. This approach offers a novel strategy for successful pancreatic islet transplantation and may be relevant to our understanding of the mechanisms involved in the development of tolerance.

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LFA-3, CD44, and CD45: Physiologic Triggers of Human Monocyte TNF and IL-1 Release

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The monocyte-derived cytokines, tumor necrosis factor–α (TNF-α) and interleukin-1β (IL-1β), are central regulators of the immune response, but the physiologic stimuli for their release remain largely undefined. Engagement of three monocyte glycoproteins, LFA-3, CD44, and CD45, by specific monoclonal antibodies immobilized on plastic induced TNF-α and IL-1β release. In addition, TNF-α was released when monocyte LFA-3 bound immobilized, purified CD2, which is its physiologic receptor. Thus, a receptor-ligand interaction that mediates cell-cell adhesion can transmit the necessary signals for the release of monokines.

MONOCYTES PRODUCE A VARIETY of soluble mediators (monokines) that can influence surrounding cells. Among such mediators are TNF-α and IL-1β, both of which are important in the initiation and regulation of the immune response (*1, 2*). Although monocytes release TNF-α and IL-1β after stimulation with endotoxin, phorbol myristate acetate (PMA), or Gram-positive bacterial components (*2, 3*), other more physiologic mechanisms for induction of monokine release must also exist, because monokine production can be induced under conditions that exclude these factors. One such physiologic mechanism may be via cell-cell contact. For example, TNF-α is elicited when monocytes contact tumor cells (*4*), and IL-1β, which facilitates T cell proliferation, is released during antigen presentation to T cells (*2*). We therefore attempted to define the mechanisms by which monocyte interaction with other cells might induce monokine release.

Receptor-ligand interactions between surface molecules on monocytes and other cells are likely to trigger this release of monokines. The binding of a monocyte surface receptor with specific monoclonal antibody (MAb) immobilized on plastic might mimic the effects of receptor engagement with its

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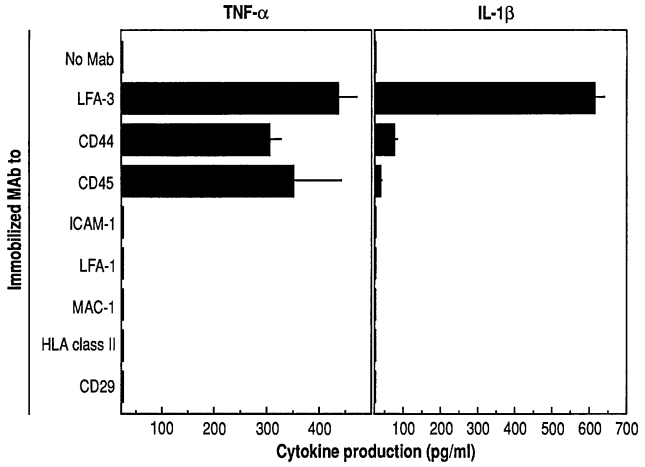

Fig. 1. Antibodies to LFA-3, CD44, and CD45 stimulate TNF-α and IL-1β production by human monocytes. The indicated MAb (10 μg/ml) was added to 96-well microtiter plates in phosphate-buffered saline (PBS; 50 μl), incubated 6 hours at 4°C, and unbound MAb removed by washing twice with PBS. The wells were then filled with RPMI 1640 containing fetal bovine serum (FBS, 10%), 2 mM glutamine, and gentamicin (0.1 mg/ml). Monocytes, purified by elutriation (*26*), were added at a concentration of 4×10^5 cells per well to give a total volume of 200 μl, and the plates were incubated overnight at 37°C in 5% CO_2. Supernatants were collected and assayed by enzyme-linked immunosorbant assay (ELISA; Cistron, Pine Brook, New Jersey) for TNF-α or IL-1β. The lowest detectable concentration of TNF-α or IL-1β was 25 pg/ml. The following MAbs were purified immunoglobulins derived from ascites unless otherwise indicated: CD44 NIH44-1 (*13*), LFA-3 MAb TS2/9 (*27*), ICAM-1 MAb 84H10 (*28*), LFA-1 MAb MHM24 (*29*), human lymphocyte antigen (HLA) class II MAb IVA12, CD29 MAb 4B4 (Coulter Electronics, Hialeah, Florida), CD45 MAb NIH45-2, and MAC-1 MAb NIH11b-1. All MAbs are of the immunoglobulin G1 (IgG1) subclass. Results presented are the mean of triplicates + SEM. Endotoxin (500 ng/ml) was used as a positive control and induced the release of 1285 pg of TNF-α and 6400 pg of IL-1β per milliliter.

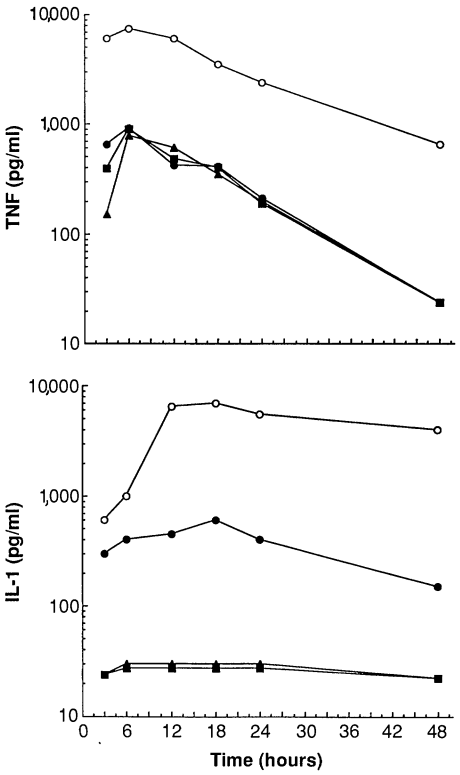

Fig. 3. The kinetics of TNF-α and IL-1β secretion by human monocytes stimulated by antibodies to LFA-3, CD44, and CD45. Monocytes were added to wells that had been previously coated with MAb to LFA-3 (TS2/9) (●), CD44 (NIH44-1) (▲), or CD45 (NIH45-2) (■), at a concentration of 4×10^5 per well and incubated at 37°C. To some wells, endotoxin (500 ng/ml) (○) was added. Supernatants were collected from different wells at 3, 6, 12, 18, 24, and 48 hours and assayed by ELISA for the presence of TNF-α and IL-1β. There was no detectable TNF-α or IL-1β release from untreated monocytes at any time. The lowest detectable concentration of TNF-α and IL-1β was 25 pg/ml. These data are from one of four representative experiments. Monocytes from different donors were used and results were similar.

ligand on another cell. We therefore surveyed MAbs to eight monocyte surface receptors for their capacity to induce TNF-α and IL-1β release from monocytes (Fig. 1). All the receptors studied are involved in adhesion or activation of leukocytes (*5–9*). Monoclonal antibodies specific for the molecules CD44, CD45, and LFA-3 (lymphocyte function-associated antigen-3) reproducibly induced TNF-α and IL-1β release from monocytes; MAbs to the other five receptors induced no detectable release of these monokines. All the MAbs listed in Fig. 1 bound to monocyte surface antigens (as determined by immunofluorescence) and appeared to facilitate binding of the monocytes to the plates (*10*). Whereas LFA-3, CD44, and CD45 MAb-induced TNF-α, only the LFA-3 MAb induced significant amounts of IL-1β release.

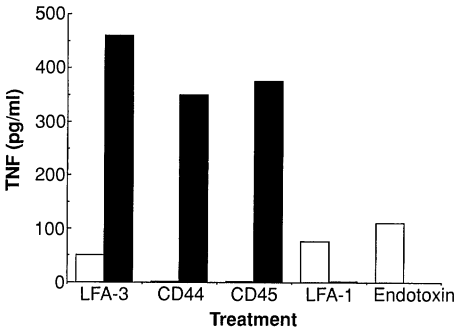

Fig. 2. Soluble antibodies do not stimulate TNF-α or IL-1β secretion. Monocytes (4×10^5 per well) were added to wells that were either previously coated with MAb or endotoxin (2 ng/ml) for 6 hours (black bars), or to which soluble MAb or endotoxin was added (white bars). After an overnight incubation, the supernatants were assayed for the presence of TNF-α. Untreated monocytes released no detectable TNF-α. The lowest detectable TNF-α concentration was 25 pg/ml. This is one of three representative experiments. Monocytes from different donors were used, and results were comparable.

The three molecules that induced monokine release are structurally unrelated. LFA-3 is part of the immunoglobulin supergene family (*11*), CD44 is related to cartilage link protein (*12*), and CD45 is a member of a distinct family of cell surface tyrosine phosphatases (*6*). Nevertheless, our findings fit concepts of these molecules emerging from other model systems. CD44 (also known as Hermes, phagocytic glycoprotein-1, or extracellular matrix receptor III) not only participates in T cell adhesion to erythrocytes (*13, 14*) and endothelial cells (*15*), but also facilitates T cell activation by its interactions with both T cells and monocytes (*13, 16, 17*). CD45 (T200, leukocyte common antigen) is an abundant molecule found on all hematopoietic cells that can modify activation of T cells in many model systems (*7*) and can mediate adhesion in at least one system (*18*). Finally, LFA-3 is a relatively ubiquitous cell surface molecule that is the ligand for the T cell protein, CD2 (*19*). The CD2–LFA-3 interaction is one of the major pathways that mediates interaction of T cells with other cells (*8, 9, 20*).

Because monocytes are so sensitive to activation by bacterial lipopolysaccharides (endotoxin), great care was taken to exclude endotoxin as a cause of these effects. Endotoxin concentrations [measured by a limulus amebocyte lysate assay (*21*)] were low or undetectable in the MAb preparations used in this study; the endotoxin concentration was <0.006 ng/ml in most of the MAb preparations. The only measurable endotoxin was found in the LFA-3 and LFA-1 MAb preparations: MAb at 10 μg/ml contained endotoxin at approximately 0.5 ng/ml. We compared soluble MAb to MAb bound to plastic for induction of monokine release (Fig. 2). When added directly to monocytes in solution, weak induction of TNF-α release that could be attributed to endotoxin was observed in the two preparations with measurable endotoxin; in contrast, after immobilization of the MAb on plates, the endotoxin appears to have been washed away, whereas the marked monokine induction by LFA-3, CD44, and CD45 MAb was revealed. Finally, we observed no induction of TNF-α release when monocytes were added to microtiter wells that had been

previously coated for 6 hours with 2 ng per milliliter endotoxin, a concentration that induces TNF-α release when added directly to the monocytes.

We found that none of the endotoxin-free MAbs were able to induce monokine release when not bound to plastic, indicating that they do not induce monokine release by binding to a specific epitope, but act by cross-linking the antigens. All MAbs used in Fig. 1 bound to the plastic and thereby mediated the attachment of monocytes to the wells (*10*); however, only those specific for LFA-3, CD44, or CD45 induced monokine release. In contrast, a recent report indicates that soluble CD44 MAb can induce release of IL-1 from human monocytes (*17*). It is unclear why this soluble MAb induces a response; however, contaminating endotoxin (cited as <1 ng/ml) may be sufficient to induce monokine release. Our data indicate that endotoxin was not a factor and that the MAbs used had to cross-link either the LFA-3, CD44, or CD45 molecules on the surface of monocytes for induction of monokine release.

For monokine release to be relevant to the early events of T cell activation, release should be rapid. Secretion of TNF-α and IL-1β was extensive within 3 hours and peaked at about 6 hours (Fig. 3). These findings are in agreement with another report (*22*). Triggering by these molecules most likely induces transcription, since similarly prepared monocytes incubated for 4 hours in media alone showed no mRNA for either IL-1 or TNF (*23*).

Interaction of T cell CD2 with LFA-3 on others cells mediates adhesion and enhances T cell responsiveness to other stimuli (*24*). The induction of monokine release by LFA-3 MAb suggests that the monocyte can, likewise, be activated by CD2 on the T cell. Therefore we determined whether purified CD2 could induce monokine secretion (Fig. 4). The results show that purified CD2 stimulates TNF-α release. The specificity of the CD2-induced release is indicated by the ability of a CD2 MAb, but no other MAb, to block the induced TNF release, and the purity of the CD2 preparation on a silver-stained SDS-polyacrylamide gel (only one band was observed) (*25*). Furthermore, a comparably purified protein, ICAM-1, failed to induce TNF release. Together, these data suggest that this response can occur in the absence of MAbs, indicating that the Fc portion of the MAbs is probably not contributing to induction on the monokines. Thus, it appears that binding of CD2 and LFA-3 transduces signals to both interacting cells.

LFA-3 and at least two other molecules, CD44 and CD45, are involved in induction of monokine release. It is unclear whether these molecules act independently or are associated functionally. We await a detailed understanding of the intracellular mechanisms whereby these molecules trigger monokine release under physiologic conditions.

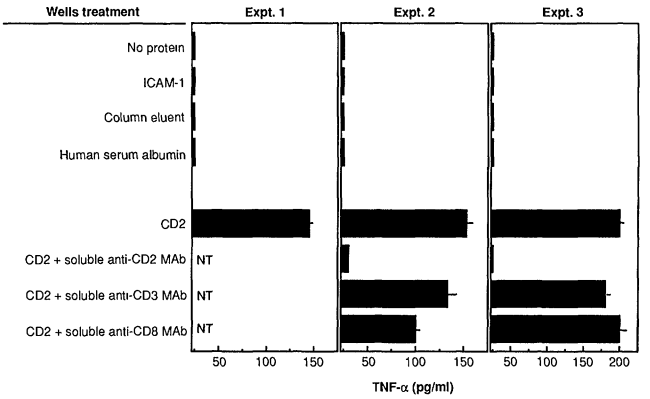

Fig. 4. Stimulation of TNF-α secretion from human monocytes by purified CD2. CD2 and ICAM-1 were purified as described (*25, 30, 31*). Microtiter wells were coated with approximately 300 ng/ml of either CD2, ICAM-1, human serum albumin, or a 1:2 dilution of a CD2-negative control fraction of the CD2 affinity column eluent for 6 hours at 4°C. The plate was then washed and monocytes were added (4×10^5 cells per well). After a 6-hour incubation at 37°C with 5% CO_2, the supernatants were collected and the concentration of TNF-α in the supernatants determined. To some wells the following MAbs were added at approximately 10 μg/ml: CD2 MAb 9.6; CD3 MAb OKT3 (American Type Culture Collection); and CD8 MAb B9.8. All three MAbs contained no detectable endotoxin. The lowest detectable concentration of TNF-α was 25 pg/ml. Results from three representative experiments are presented. Values are the mean of duplicates (+ SEM). NT, not tested.

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25. CD2 was purified by solubilizing Jurkat cells in lysis buffer containing 1% Triton X-100 and protease inhibitors. The lysate was fractionated through an affinity column of the CD2 MAb 9.6 coupled to Sepharose beads. CD2 eluted in a single peak with glycine, pH 2.75. The CD2 preparation gave a single diffuse band of 50 to 60 kD on a 8 to 16% SDS–polyacrylamide gradient gel by silver staining and contained endotoxin at <12 pg/ml.
26. Human mononuclear cells were obtained from healthy adult donors by leukopheresis and isolated on a Ficoll-Hypaque (Pharmacia Fine Chemicals). Purified monocytes were obtained by counter current elutriation. The resulting cell population was always ≥95% monocytes as determined by Giemsa-stained preparations and flow cytometry.
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