New Approaches to Transplant Immunosuppression

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ABSTRACT

Although considerable progress has been achieved using immunosuppressive drugs that inhibit lymphocyte activation and T-cell cytokine signal transduction pathways, the widespread tissue distribution of the molecular targets exploited to date, calcineurin, mammalian target of rapamycin, and inosine monophosphate dehydrogenase, engenders a constellation of collateral toxicities. One strategy to develop new immunosuppressants seeks to identify targets that are critical for and specific to the adaptive immune response. Three approaches have been used to guide this enterprise; molecular design based on steric resemblance of the antagonist to the natural ligand; construction of complementary DNA oligonucleotides that hybridize with the leader sequence of messenger RNA encoding the synthesis of the specific target, thereby preventing production of that protein; and functional comparisons based on similar inhibitory profiles of candidate compounds and a probe that blocks the target nonselectively. Use of these 3 technologies has led to identification of antagonists blocking selectins, intercellular adhesion molecule-1, or Janus kinase 3, respectively. These lead compounds have been tested for their effects on the alloimmune response and/or the ischemia-reperfusion injuries.

Although the development of azathioprine, an imidazole derivative of 6-mercaptopurine, was based on chemical modifications of the purine adenine, recent discoveries of immunosuppressive agents have depended on serendipity. Compounds isolated from soil microbial samples were incidentally discovered to block lymphocyte activation. For example, cyclosporine, isolated from Tolypocladium inflatum Gams, was demonstrated by Borel et al to be immunosuppressive after it had failed tests as an antibacterial agent. Also, sirolimus, isolated from Streptomyces hygroscopicus, did not fulfill its original intent as an anticandidal agent; rather, Sehgal showed it to be a potent antiproliferative drug. Similarly, serendipity fostered the documentation of the immunosuppressive properties of tacrolimus, mycophenolic acid, and FTY720 by Goto et al, Allison and Eugui, and Chiba et al, respectively.

While cell surface membrane molecules have been targeted by the production of specific antibodies, particularly monoclonal antibodies, and by the synthesis of receptor-immunoglobulin conjugates, the possibility of host antireagent neutralizing responses, the requirement for parental administration, as well as the high production costs have precluded the application of these agents for long-term therapy in humans. In contrast, new and more sophisticated approaches exploit steric, sequence, or function principles to identify small molecules that display therapeutically actions targeted toward elements that participate in allograft rejection and/or the ischemia-reperfusion injuries.

The first strategy seeks to model the antagonist using steric analysis of the active site of the target molecule. Using crystallographic tools to visualize the interaction of Sialyl-Lewisx with leukocyte (L-), endothelial (E-), and platelet (P-) selectins, Kogan et al designed a nonpeptidic glycol compound, Tbc 1269 (Texas Biotechnology Corporation, Houston, Texas, United States). The antagonist competitively binds to all 3 selectins at concentrations 2- to 8-fold lower than those necessary for the natural ligand. Direct intraarterial perfusion (4 mg) or continuous peripheral intravenous (IV) infusion (20 mg/kg/d) of Tbc 1269 has been shown to improve the renal function of ischemially damaged Lewis rat isografts and to mitigate alloimmune

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responses toward Lewis kidneys in major histocompatibility complex (MHC)-incompatible AC1 hosts.\textsuperscript{8} However, the steric approach has not proved fruitful to design antagonists of enzyme action; most notably, an intense crystallographic analysis failed to achieve a potent, biologically active, more selective, structural analogue of tacrolimus.

A second elegant approach to the design of antagonists for a specific target is the antisense oligonucleotide strategy. The short (20-mer) sequence of DNA encoded in ISIS 2302 (ISIS Pharmaceuticals, Carlsbad, Calif, United States) is complementary to a portion of the leader sequence (3'-untranslated region) of human intercellular adhesion molecule-1 (ICAM-1) messenger RNA (mRNA). ICAM-1 was selected as the target because it participates both in the ischemia-reperfusion injury as an endothelial cell marker that attracts leukocytes bearing lymphocyte function antigen-1 (LFA-1) and in the alloimmune response by acting as a coreceptor on antigen-presenting elements that interact with LFA-1 on immunocompetent cells. Following entry into the cell, the oligonucleotides bind to the complementary target mRNAs. The newly generated double-stranded nucleic acid in the cytoplasm provokes cleavage of the specific ICAM-1 message by RNase H, resulting in failure of translation and of ICAM-1 protein production. To prolong the half-life of these oligonucleotides in the circulation, backbone phosphates are substituted with sulfur atoms, producing phosphorothioates.

Either on intraarterial perfusion of kidneys (10 mg) or on continuous IV delivery (20 mg/kg/d), ICAM-1 antisense molecules interrupt both processes.\textsuperscript{9} Although a preliminary Phase I/II trial showed the agent to be well tolerated, the ISIS 2302 phosphorothioate neither improved initial renal function nor reduced the incidence of acute rejection episodes.\textsuperscript{10} This failure has been attributed to poor penetration of plasma membranes due to the high negative charge of the ISIS 2302 phosphorothioate molecules. This limitation has been addressed in animal models by encapsulation in liposomes; however, this strategy may be problematic in clinical settings due to the likelihood that penetration of liposomes is likely to serve as an adjuvant to intensify the host immune response.

Recently, Stepkowski et al\textsuperscript{11} demonstrated an extended half-life and increased cell penetration when the 7 terminal residues in the 3'-end of the oligonucleotide were substituted with neutral methoxyethyl groups, thereby reducing the impact of the highly charged phosphorothioate groups. These methoxyethyl oligonucleotides show a 2.5-fold enhanced protection against the ischemia-reperfusion injury and a 2-fold increased prolongation of allograft survival compared with the phosphorothioate oligonucleotides. Furthermore, they can be delivered either by IV infusion or by the oral route, which makes them more amenable to clinical trials than the phosphorothioates, which had to be delivered by IV infusion.

The “function” strategy seeks to identify a candidate drug that acts as a selective inhibitor of a target molecule by using biologic analyses of its in vitro effects on 60 human tumor cell lines, including those from leukemia, melanoma, and cancers of the lung, colon, breast, brain, ovary, prostate, and kidney. First, the investigator identifies a “probe” compound that blockades the target, even if it is a nonexclusive action, that is, it inhibits not only the desired target but also other enzymes displaying similar activities. The “signature” of the probe is determined by testing it on the 60 cell lines to assess its pattern of 50% growth inhibition (GI50), total growth inhibition (TGI), and 50% reduction in protein indicating the net loss of cells (LC50).\textsuperscript{12} The mean graph signature is the pattern created by plotting positive and negative values, which are generated from the set of GI50, TGI, or LC50 values, with reference to a vertical line that represents the mean response of all the cell lines to the test probe. The National Cancer Institute maintains a file of the signatures of 40,000 compounds. Using a pattern-recognition algorithm, COMPARE, this file is searched using pairwise correlation coefficients between the probe and the other compounds.

The target molecule that we selected is Janus kinase 3 (Jak3), a tyrosine kinase that interacts with the \( \gamma \)-common chain of cytokine receptors of the interleukin-2 (IL-2) family, including IL-2, -4, -7, -9, -13, and -15. Jak3 mediates T-cell activation by phosphorylating the \( \gamma \)-common chain and the 2 signal transducers and activators of transcription-5a/b (STAT5). The action of Jak3 is critical for T-, B-, and natural killer (NK) cell activities. Furthermore, the enzyme is restricted to a lymphoid tissue distribution.

Using the COMPARE program, we sought to identify a lead compound on the basis of the hypothesis that comparable mean graph signatures signify similar mechanisms of action. The probe for this enterprise was the tyrophostin family member and analogue of erbsstatin, AG490 (C\(_{17}\)H\(_{14}\)N\(_2\)O\(_3\)),\textsuperscript{13} which we had previously showed to inhibit Jak3 in addition to its action to block the closely related analogue Jak2, a widely distributed tyrosine kinase that participates in many signal transduction processes. Using the COMPARE program, we identified 25 compounds that display a Pearson’s correlation coefficient of 0.70 with the signature of AG450. These potential Jak3 antagonists were then tested for selectivity of inhibition using the rat lymphoid Nb 2 cell line, which responds to IL-2 stimulation via the Jak3 pathway or to prolactin stimulation via the Jak2 pathway. Inhibition of the former but not the latter response by a drug provided the criterion of selectivity to identify a lead compound among the 25 candidates.

NC1153 shows a 2.5-fold window between Jak3 versus Jak2 inhibition. The compound inhibits the growth of activated but not nonactivated Jak3-positive T cells and has no effect on Jak3-negative Jurkat cells. Not only does the compound show little inhibition of Jak2, but also it does not block the generation of signal 1 intermediates, namely, phosphorylation of either Zeta-associated protein 70 or p56\(^{ll\kappa}\) as well as synthesis of the IL-2R\(\alpha\) chain. When administered by the IV or oral route, NC1153 prolongs allograft survival and shows synergistic immunosuppressive actions with the signal 1 inhibitor cyclosporine. Preliminary
findings support the hypothesis that NC1153 is free of collateral nephrotoxic, myelosuppressive, and lipotoxic side effects.

Ongoing research efforts are combining these approaches to refine existing reagents, namely, the application of steric approaches to develop a second-generation inhibitor initially identified by the function approach. The goal of this enterprise is improved immunosuppressive efficacy with minimized collateral toxicities, thereby improving the morbidity associated with transplantation therapies.

REFERENCES