FTY720: A New Dimension in Transplantation

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In contrast to the subfamily of neutrophil and mononuclear chemotactic cytokines (chemokines) up-regulated by inflammation, FTY720 seems to affect the interactions of lymphocytes with the subfamily of homing chemokines, secondary lymphoid tissue chemokine (SLC) and essential myosin light chain chemokine (ELC). These homing chemokines are constitutively expressed in T-cell zones of secondary lymphoid structures (SLS), including peripheral lymph nodes (PLN), Peyer’s patches (PP), appendix, and spleen (SPL), wherein they attract lymphocytes and dendritic cells bearing the corresponding receptors CCR7 or CXCR3 (SLC) or only CCR7 (ELC).

The chemokine receptor CCR7 is expressed on stomal elements within T-cell zones, on dendritic cells (DC), on B cells, on T lymphocytes bearing the Th1 as opposed to Th2 phenotype, and on memory cells remaining within SLS in contrast to the circulating effectors that generate inflammatory mediators. (CCR7 is not present on granulocytes or monocytes.)

Following binding of CCR7 to ELC and SLC, lymphocytes display activation of integrin adhesion molecules, events necessary for incorporation into the critical microenvironment for immune maturation. Indeed, cells from CCR7 knockout (KO) mice fail to develop delayed-type hypersensitivity reactions or primary antibody responses.

ROLE OF CHEMOKINES IN ALLOIMMUNITY

A complex pattern of inflammatory chemokines is generated during alloimmune responses. During the early phases of acute rejection in rodent allografts, the T-lymphocyte chemoattractants, lymphotactin and SINC/KC, show augmented rates of production. Thereafter, both RANTES (regulated on activation T cell expressed and secreted), a marker of T-cell infiltration, and interferon (IFN)-inducible protein-10 (IP-10) are increased in rodent renal and in human cardiac grafts. In human liver transplant rejection, macrophage inflammatory protein (MIP)-1β and MIP-1α are increased, events that potentiate the activating effects of IFN-γ on mouse macrophage cytokine production and antagonize Th2 cytokines. Correspondingly, human renal allografts undergoing rejection display infiltrating mononuclear cells bearing the G-protein-coupled receptors (GPCR) CXCR4 and CCR5.

The pleiotropic expression of MIP-1α/β, monocyte chemoattractant protein (MCP)-1, RANTES, and IP-10, as well as their corresponding receptors, namely, CCR1 (MIP-1α), CCR2 (MCP-1), CCR5 (MIP-1α/β, RANTES), and CXCR3 (IP-10), has been confirmed in rejecting major histocompatibility complex (MHC)-mismatched murine cardiac allografts. These authors observed that CCR1−/− KO mice did not reject class II-mismatched allografts, an effect that was augmented by cyclosporine (CsA) treatment. In other studies, sirolimus (SRL) was reported to decrease KC, MIP-2, and IL-8 production, as well as polymorphonuclear leukocyte infiltration into rat cardiac allografts.

Chronic rejection and emergence of transplant-associated accelerated atherosclerosis have also been associated with persistence of RANTES, as well as increased MIP-1 and MCP-1. The activity of the latter two chemokines suggests a critical role for intragraft macrophages, a hypothesis supported by the apparent benefit produced by gamma lactone, which inhibits macrophage effector activation.

FTY720: A PUTATIVE CHEMOKINE RECEPTOR AGONIST

FTY720, 2-amino-2-[2-(4-octylphenyl-ethyl)]-1, 3-propanediol, a synthetic analogue of myriocin, reversibly depletes peripheral blood lymphocytes (PBL) but not granulocytes or monocytes.

Shinomiya et al suggested that FTY720 induces lymphocyte apoptosis. They attributed this effect to activation of phospholipase C (PLC), mobilization of calcium, and up-regulation of caspase-1 and caspase-3. However, these observations were made using massive exposure to the agent and, therefore, may not be relevant to the immunosuppressive effects observed at clinical doses of 1.0 to 5.0 mg per day. At immunosuppressive doses, FTY720 and vehicle-treated transplant recipients showed similar frequencies of cytotoxic ACI anti-LEW (CTL) precursors in limiting-dilution analyses of PLN and SPL lymphocytes and equal staining for apoptotic cells in allografts and PLNs.
using the TdT-mediated deoxyuridine 5' triphosphate nick-end labeling (TUNEL) assay (data not shown).

Alternatively, Chiba and colleagues suggest that FTY720 produces a reversible effect to sequester lymphocytes to SLS. To confirm this hypothesis, we observed that purified lymphocytes labeled with 5-6-carboxylfluorescein diacetate succinimide ester (CF) upon adoptive transfer from FTY720-treated donors into syngeneic Balb/c hosts showed reduced numbers of CF\(^{+}\) cells (\(10^{3} \pm SD\)) in the blood (\(P < .03\)) and increased numbers in mesenteric lymph nodes (MLN), PP, and, to a lesser extent, PLN. Neither SPL nor thymus (TY) showed a significant increase in lymphocyte sequestration. Furthermore, transfers of purified CD4\(^{+}\) or CD8\(^{+}\) T subsets as well as B cells showed similar migration patterns as the Pan-T cell population.

It has been observed that the lymphocyte sequestration effect of FTY720 is sensitive to Bordetella pertussis toxin and that the up-regulated chemotactic responses to SLC/ELC in vitro demand expression not only of the CCR7, but also of the Edg6 receptor. First, it was shown that FTY720 enhanced in vitro chemotaxis. Second, the in vivo sequestration effects produced by FTY720 were markedly reduced by administration of Bordetella pertussis toxin, an agent that inhibits Go, ADP ribosylation of the Go subunit of heterotrimeric G-proteins. Five further observations support the hypothesis that the lymphocyte sequestration effect produced by FTY720 depends upon CCR7-mediated adhesion: (1) abrogation by anti-CCR7 antibody; (2) modulated effect in plt-/plt- mice, which have a deletion of SLC and a 70% to 80% reduction in ELC; (3) transfection of Edg6 and CCR7 (but not one marker alone) conferred upon HEK293T cells the distinctive pattern of in vitro chemotactic responsiveness elicited by FTY720; (4) Clostridium toxin B (100 ng/mL), an agent that blocks the Rho family molecules, partially abrogated the chemotactic effect of double Edg6/CCR7 transfectants; and (5) FTY720 enhanced actin polymerization by HEK2397T•Edg6 cells.

EDG RECEPTOR SIGNALING PATHWAYS LEADING TO CCR7 UP-REGULATION

Because FTY720 is a structural analogue of sphingosine, it seems reasonable to assume that it acts as a profound agonist for Edg6, a member of the family of receptors (Edg1, -3, -5, -6, and -8) that bind sphingosine-1-phosphate (SIP), in contradistinction to the Edg2, -4, and -7 family with receptors for lysophosphatidic acid (LPA, 1-acylglycerol-3-phosphate). Edg6 has limited distribution on lymphocytes and in lung tissue with receptors for lysophosphatidic acid (LPA, 1-acylglycerol-3-phosphate). Edg6 has limited distribution on lymphocytes and in lung tissue with receptors for lysophosphatidic acid (LPA, 1-acylglycerol-3-phosphate).

The preclinical results on lymphocyte homing were confirmed by a multiple-dose Phase I clinical study reported by the applicant group. Ascending amounts of FTY720 added to the CsA-Pred regimen of cohorts of stable renal transplant patients produced a prompt, dose-dependent, and reversible decrease in the number of circulating PBLs without effects on monocytes or neutrophils. Another multicenter, randomized, open-label study evaluated the safety, tolerability, and efficacy of FTY720 versus mycophenolate mofetil (MMF) with CsA and steroids in de novo transplantation. Episodes of transient bradycardia without symptoms or sequelae, most of which occurred within the first 24 hours posttransplant, were reported in 11/124 (8.9%) of FTY720-treated patients versus 2/35 (5.7%) of MMF-treated patients. Graft survival was 99% (one graft loss in the MF group) and patient survival was 100%. FTY720 was well tolerated and appeared to be effective in the prevention of acute rejection in combination with CsA and steroids.

In addition, immunosuppressive regimens that do not include a calcineurin antagonist are of especial interest for patients at increased risk of delayed graft function (DGF). Early results in an ongoing study suggest the potential utility of an FTY720/RAD regimen in the setting of such
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high-risk renal transplant patients. Entry criteria included a constellation of risk factors for DGF defined as the need for a dialysis treatment, namely, retransplant status, African-American ethnicity, prolonged cold ischemia time, advanced donor age, and cause of donor death due to cerebrovascular accident. The immunosuppressive regimen included FTY720 (loading dose 4 mg, maintenance dose 2.5 mg/d), everolimus according to a concentration-controlled regimen (6 to 8 ng/mL trough), and tapering doses of steroids. Follow-up data are available for 11 to 93 days posttransplant (mean 96 days). As predicted, virtually every patient experienced DGF. During the follow-up period, six (22%) patients experienced an acute rejection episode; three (11%), graft loss; and one (3%), death.

SUMMARY

FTY720 is a myriocin derivative currently undergoing multicenter trials for the prophylaxis of renal transplant rejection. The drug is a novel immunomodulator demonstrating unique and potent pharmacologic activity by selectively altering lymphocyte homing from peripheral blood to SLS. Initial clinical trials indicate that FTY720 absorption and disposition is predictable and dose-linear, with minimal circadian variation. The time needed to reach steady state is long, suggesting the benefit of a loading dose. Doses up to 5.0 mg/d for 28 days were well tolerated in stable renal transplant patients maintained on cyclosporine (CsA) and steroids, with no evidence of a pharmacokinetic interaction with CsA. A combination regimen (FTY720, CsA, and steroids) reduced the occurrence of acute rejection episodes to less than 10% in a pilot study of de novo renal transplant patients. Preliminary data from another pilot study using the alternate combination (FTY720, RAD, and steroids) also shows a marked decrease in these episodes in high-risk patients. The unique mode of action, independence from cytochrome P450 3A4 metabolism, and suggestions of immunosuppressive potency together suggest that FTY720 will be a valuable addition to the immunosuppressive armamentarium.

REFERENCES