

## **BASIC SCIENCE REVIEW FOR THE PEDIATRIC RHEUMATOLOGIST**

### **Up and coming therapeutics: FTY720**

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Adaptive immune responses take place in the secondary lymphoid organs of the body. The secondary lymphoid organs include the spleen, lymph nodes, tonsils, and Peyer's patches. T and B lymphocytes gather in these organs and survey for incoming antigens that they recognize. Unlike cells of most organs, however, the majority of the lymphocytes are not permanent residents of the lymphoid tissues. Instead, to maximize the chance of encountering the right antigen, T and B cells constantly recirculate through the body. In the case of peripheral lymph nodes, B and T cells enter from the bloodstream into the parenchyma of the lymph node via high endothelial venules and, within the lymph node, home into their respective zones by following chemotactic cues. In the absence of activation by antigen encounter, the T and B cells will pause in a particular lymph node for only about a day. They will then exit the lymph node via efferent lymphatics and are eventually returned to the blood circulation via the thoracic duct. Once in the circulation, they are able to enter a different lymphoid tissue to further survey for antigens.

If T cells are activated in the lymph node, however, they differentiate into effector cells that leave the lymph node and home to the peripheral tissues [reviewed in 1, 2]. The ability to mount efficient immune responses, then, is dependent on the ability of lymphocytes to recirculate through the body to find their cognate antigens and for the effector cells to leave the lymph node to home to peripheral tissues. Sequestration of lymphocytes within the lymph node, then, could be a potential method of immune suppression.

In 1994, a compound, ISP-1, was isolated from a fungus used in Chinese herbal medicine. This compound had immunosuppressive activities that were 10 to 100-fold more potent than cyclosporine, which had also been isolated from the same fungus. ISP-1 inhibited serine palmitoyltransferase and demonstrated anti-proliferative properties. In search of a more potent derivative with fewer side effects, FTY720 was synthesized in 2000 [3]. FTY720 demonstrated potent immune suppression. In vivo, it effectively prolonged graft survival in preclinical models of organ graft rejection and graft versus host disease [4, 5]. In adjuvant

and collagen-induced models of arthritis, FTY720 was able to ameliorate paw swelling and bone destruction [6].

Unlike ISP-1, FTY720 did not inhibit serine palmitoyltransferase. Instead, investigators noticed that it reduced the number of circulating T and B cells in the bloodstream. Although cytotoxic in micromolar concentrations *in vitro*, the effect on blood lymphocyte counts occurred at nanomolar concentrations in the bloodstream. Since cytotoxicity was unlikely, Chiba et al looked at lymphoid tissue counts of lymphocytes and found that FTY720 treatment lead to a rapid increase of lymphocytes within lymphoid tissues that corresponded to the decline in blood counts and thoracic duct lymphocyte counts. FTY720, then, caused a sequestration of lymphocytes within lymphoid tissues, and they hypothesized that the immunosuppressive activity of FTY720 was related to the defective lymphocyte circulation [7].

The mechanism for the FTY720-mediated sequestration was partially elucidated in 2002 by Mandala et al. [8]. They found that FTY resembled the lysophospholipid sphingosine and that the drug was metabolized into a compound that resembled sphingosine-1-phosphate (S1P). Competitive binding assays showed that the metabolized FTY720 could bind four of five S1P receptors and *in-vitro* assays showed that FTY720 could act as an S1P receptor agonist. *In vivo*, FTY720 treatment or S1P infusion resulted in the rapid induction of lymphopenia in blood. The investigators also examined thoracic duct cell counts as a measure of lymphocyte retention in lymphoid tissues, and found a relative lymphopenia there as well. These results showed that FTY720 could act as an S1P agonist *in vivo*, and also revealed S1P to be a mechanism for mediating lymphocyte sequestration within lymphoid tissues. Further analysis revealed that S1P seemed to act at the point of lymphocyte egress from lymphoid tissue into lymphatic sinuses, although the relative contributions of the lymphocytes versus the sinus endothelium were unclear.

More recently, analysis of S1P receptor knockout mice revealed that mice missing a single S1P receptor, S1P1, mimicked the phenotype associate with FTY720 treatment. Moreover, the absence of S1P1 on the lymphocytes alone in

the presence of normal S1P1 on the sinus endothelium, could mediate the effect, suggesting that the control of lymphocyte egress was mediated by lymphocyte, rather than endothelial, factors. Because the S1P1-deficient mice resembled FTY-treated mice and because FTY treatment led to a down-regulation of lymphocyte S1P1, the authors hypothesized that FTY may act as a partial antagonist to S1P1 [9].

How the blockade of lymphocyte exit from the lymph node results in clinically useful immunosuppression is not yet well understood, but studies have been suggestive of several mechanisms. Both naïve and activated/effector T cells are sequestered from the bloodstream [10, 11]. Using a delayed hypersensitivity response and an autoimmune diabetes model to test for effector CD8 activity, Pinschewer et al showed that the sequestration of these cells by FTY720 was associated with attenuated end organ damage [12]. The inability of effector cells to leave the lymph nodes and home to end organs, then, may be one of the reasons why FTY720 has been effective in models of solid organ transplant. The sequestration of naïve T lymphocytes was also associated with decreased local (i.e. draining lymph node) primary immune responses, which may also contribute to useful clinical immune suppression [11]. Interestingly, immune responses to models of systemic infection are preserved [12]. Potentially, this attribute may be very useful clinically. This type of immune response is mediated primarily by the spleen, which has a circulation pattern distinct from that of lymph nodes [1]. Further investigations are needed to understand how FTY differentially affects different types of immune responses.

Recently, FTY was tested in a multiple-dose, randomized, placebo-controlled phase I study of renal transplant patients. As expected, FTY720 administration to stable renal transplant patients maintained on cyclosporine and prednisone resulted in a dose-dependent transient drop in peripheral lymphocyte counts. The drug was well tolerated for up to 28 days except for asymptomatic bradycardic episodes and did not affect blood concentrations of cyclosporine. Importantly, the incidence of infections was similar between placebo and treatment groups [13]. Novartis, the manufacturer of FTY, is also conducting a

small pilot study on multiple sclerosis patients (Shreeram Aradhye, Novartis, personal communication). The question for us is whether FTY will be useful for the treatment of our autoimmune diseases. The utility of FTY720 for complex systemic diseases such as SLE will need to await further investigation of FTY720 as well as of the anatomical origin of these diseases. If the drug truly does work by preventing naïve cells from recirculating or effector cells from reaching their target, FTY720 could be potentially useful in antigen-driven T cell-mediated diseases such as juvenile arthritis [14]. This is a drug we should watch for in the near future.

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