that in some cases, 5 years of funding for projects was not long enough to deliver results. Although the foundation is known for its “lean” structure, some grantees said that current staff levels are barely adequate to handle the existing workload, and Yamada said that it will have to grow in order to double its spending. Choosing worthy recipients, monitoring projects, and measuring their effects will be especially challenging. “For our largest grants, GAVI and the Global Fund, we know the results that they’ve produced and they’re pretty substantial,” said Yamada. “For others, it’s harder to measure . . . [but] we’re beginning to get some evidence.” In Botswana, for example, where the foundation supports a national HIV–AIDS testing and treatment program, the prevalence of HIV infection among girls 15 to 19 years of age decreased by 22% between 2003 and 2005.

Perhaps the Gates Foundation’s greatest influence derives from its assumption that intractable problems can be solved, given enough money and international cooperation. For example, as a condition of receiving $287 million in grants for AIDS-vaccine research that were announced in July, 165 scientists in 19 countries will have to share their data in a central repository. Yamada predicted that such collaboration will become more common in the future, even in industry. “We’re trying to deal with very difficult problems that people are suffering from in the developing world,” he said. “The more information sharing there is, the more patients will benefit.”

Dr. Okie is a contributing editor of the journal.


FOCUS ON RESEARCH

Fingolimod and Sphingosine-1-Phosphate — Modifiers of Lymphocyte Migration

Steffen Massberg, M.D., Ph.D., and Ulrich H. von Andrian, M.D., Ph.D.

Multiple sclerosis is considered an autoimmune disease in which CD4+ T cells and macrophages destroy oligodendrocytes, which synthesize and maintain axonal myelin sheaths in the central nervous system (CNS). This misguided attack results in progressive focal demyelination that can cause severe neurologic disability. In this issue of the Journal, Kappos et al. (pages 1124–1140) report that the immunosuppressant fingolimod (also called FTY720 or 2-amino-2-(2-[4-octylphenyl]ethyl)-1,3-propanediol hydrochloride) exerted considerable therapeutic effects in a small, placebo-controlled clinical trial involving patients with relapsing multiple sclerosis. Patients who received oral fingolimod once daily had a rapid reduction in disease activity, reflected in significant reductions in the relapse rate and in the number of CNS lesions found on magnetic resonance imaging. Patients who initially received placebo also had improvement after switching to fingolimod.

Studies of the pathogenesis of autoimmune encephalomyelitis in an animal model resembling human multiple sclerosis have uncovered an essential role of T-cell migration between the blood and two anatomical compartments, the CNS and the lymph nodes.1 The disease is thought to be initiated in lymph nodes that receive lymph from the CNS. Here, oligodendrocyte-derived self-antigens are presented to T cells, which are constantly recruited to lymph nodes from the blood.

The majority of the T cells that recognize self-antigens are eliminated in their birthplace, the thymus, before they enter the systemic circulation. So in healthy persons, T cells that home to lymph nodes are either oblivious to any self-antigens that may be presented there or are not permitted to respond effectively. These naive T cells spend a day or less, on average, in the lymph nodes before they depart for the efferent lymphatics in the lymph-node medulla and then return to the blood. For reasons that are still poorly understood, patients with autoimmune diseases harbor T cells that can become activated by self-antigens.
On encountering an activating antigen on an antigen-presenting cell in the lymph node, the naive T cell begins to divide vigorously and remains sequestered in the lymph node for several days. After this period, the activated T cell has spawned an army of effector cells capable of attaching and often eradicates the source of the activating antigen. The effector cells then return to the blood by way of the efferent lymphatics and migrate to the peripheral target tissues. When they encounter the antigen again in the periphery, they are programmed to eliminate it. This response is essential for combating pathogens, but it can have dire consequences when a self-antigen (e.g., on an oligodendrocyte) is mistaken for a foreign antigen.

The clinical importance of T-cell migration in patients with multiple sclerosis has been impressively demonstrated by the therapeutic effects of Natalizumab, a monoclonal antibody that blocks the α4 integrin–dependent adhesion of blood-borne encephalitogenic T cells and macrophages to microvessels in the CNS. Fingolimod also interferes with T-cell migration, but at a different step; the drug prevents lymphocytes from leaving lymph nodes and other tissues. The sequestration of T and B lymphocytes in lymphoid tissues results in the nearly complete disappearance of lymphocytes, but not myeloid leukocytes, from the blood. This process is reversible; lymphocytes reappear in the blood after the cessation of treatment, indicating that fingolimod does not kill lymphocytes.

Fingolimod is a chemical derivative of myriocin, a metabolite of the fungus Isaria sinclairii, which has been used in Chinese traditional medicine. Although its lymphopenia-inducing effect has been recognized for more than a decade, the molecular mechanism was uncovered only recently. When fingolimod enters the bloodstream, it is rapidly phosphorylated so that it closely resembles the lyosphospholipid sphingosine-1-phosphate (S1P). S1P has five known receptors (S1P1, S1P2, S1P3, S1P4, and S1P5) that are involved in multiple physiologic activities, including neurogenesis, cardiovascular development, vasoregulation, endothelial-cell function, and leukocyte migration. Phosphorylated fingolimod binds to all S1P receptors except S1P2. S1P3, the predominant S1P receptor expressed on lymphocytes, is a major regulator of lymphocyte migration because S1P signaling is required for extravascular lymphocytes to emigrate from tissues (see diagram).

A genetic deficiency in T-cell–expressed S1P1 blocks the egress of newly generated T cells from the thymus into the blood and prevents lymphocytes in the lymph nodes from migrating into the medullary sinuses. The similar effect of fingolimod treatment indicates that its long-term effects are those of a functional antagonist. However, the precise mechanism by which fingolimod acts on S1P1 is more complex. The drug probably acts initially as an agonist of S1P1, but it results in rapid internalization and the sustained loss of the receptor’s surface expression.

When fingolimod is given to patients with multiple sclerosis, it is likely to trap newly generated encephalitogenic T cells in lymph nodes and hence prevent them from migrating to the CNS. However, part of the benefit of fingolimod could be unrelated to its effect on lymphocyte migration. For example, fingolimod has been found to modulate oligodendrocyte maturation and astroglia proliferation in vitro. Moreover, neural-tube closure is severely disturbed in mouse embryos that lack either S1P1 or two sphingosine kinases that are needed to convert sphingosine to S1P. Although the effect of these neurobiologic activities of the S1P–S1P pathway on the clinical efficacy of fingolimod in multiple sclerosis is not known, the mutant-mouse studies suggest that fingolimod should be avoided during pregnancy.

Kappos et al. report a higher incidence of adverse events in patients who received fingolimod than in those who received placebo. Common side effects included a dose-dependent transient reduction in the heart rate within hours after the first dose, increased mean arterial blood pressure, and airway obstruction. These observations are not unexpected, since S1P is involved in the regulation of the heart rate as well as vascular tone and modulates the contraction and proliferation of smooth-muscle cells in the airway. These off-target activities of fingolimod might derive from its action on S1P receptors other than S1P1. Thus, at least some of these undesired effects might be avoidable with newer compounds that target S1P1 exclusively.

Given the immunosuppressive effects of fingolimod caused by the down-regulation of S1P1, all inhibitors of this pathway will need to be evaluated for their potential to increase the patient’s susceptibility to infections. In studies of mice with acute sys-
Fingolimod and Sphingosine-1-Phosphate — Modifiers of Lymphocyte Migration

Inflammatory cells contribute to progressive tissue damage and demyelination.

Antibodies

Inflammatory lesion formation

Medullary sinus entry

Naive T cells leave the thymus in an S1P1-dependent fashion.

The S1P-S1P1 pathway plays an accessory role in homing to PLN.

Transient loss of S1P1 expression after antigen encounter.

Clonal expansion and effector differentiation of autoreactive T cells.

S1P1 re-expression when the effector cells are ready to depart.

T-cell egress from PLN requires degradation of interstitial S1P by S1P lyase.

Low S1P bioavailability in lymphoid tissue.

S1P1 on both lymphatic endothelium and T cells controls T-cell emigration.

Fingolimod

Thymus

Thymic T-cell emigration

Naive T cell

S1P

Fingolimod

HEV

Lymphocyte homing to SLO by way of HEV

Naive T cell

S1P

Fingolimod

CNS draining PLN

Efferent lymph vessel

Egress from PLN through efferent lymph vessel

Encephalitogenic T cells adhere to microvascular endothelial cells in the CNS.

Natalizumab

Recruitment of activated T cells

α4β1 integrin

Vascular-cell adhesion molecule 1 (VCAM-1)

Antigen-presenting cell (astrocyte, microglial cell)

Monocytes and macrophages

CD8+ T cell

B cell

Peripheral blood

Inflammatory lesion formation

Histotoxic mediators

Antibodies

CNS

Inflammatory cells contribute to progressive tissue damage and demyelination.

CNS draining PLN

Afferent lymph vessel draining antigen from CNS

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SIP and Fingolimod in Multiple Sclerosis.
Naive T cells (including cells that respond to self-antigens) are generated in the thymus and migrate into the systemic circulation. Blood-borne T cells continuously recirculate through peripheral lymph nodes (PLNs), which they enter by way of high endothelial venules (HEVs). Lymphocytes home to secondary lymphoid organs (SLOs) by way of HEV. In the peripheral lymph nodes of patients with multiple sclerosis (MS), autoreactive CD4+ T cells are thought to encounter antigen-presenting dendritic cells (DCs) and differentiate into effector cells. The now encephalitogenic T cells leave the peripheral lymph nodes through the medullary sinus. Once lymph-borne, encephalitogenic T cells gain access to the bloodstream and adhere to endothelial cells in the CNS, a step that is blocked by natalizumab. In the brain, T cells are reactivated and induce a detrimental inflammatory reaction. Fingolimod can interfere with several links in this chain of events. Most important, fingolimod blocks the migration of mature T cells from lymphoid organs into the lymph and blood (solid blocking symbols) and thus prevents the migration of autoreactive effector cells to the brain. In contrast, the modulation of T-cell homing to PLNs by fingolimod appears to play a secondary role (dashed blocking symbol).

virus-specific antibodies in secondary lymphoid organs, but it reduced the number of virus-specific effector lymphocytes in peripheral blood and tissues. Indeed, Kappos et al. report that patients who received the higher dose of fingolimod (5.0 mg per day) had more frequent upper respiratory tract infections than patients who received the lower dose (1.25 mg) or placebo.

Long-term suppression of lymphocyte migration to the CNS could have additional undesired consequences. For example, two recent phase 3 clinical trials showed that natalizumab (alone or in combination with interferon beta-1a) was effective in patients with relapsing multiple sclerosis. However, progressive multifocal leukoencephalopathy, a potentially fatal opportunistic infection of the CNS caused by the JC polyomavirus, developed in three patients who received natalizumab. The development of this disorder in a small number of natalizumab recipients was unexpected, but it might have been due to the drug-induced absence of immune surveillance of the CNS. Whether long-term inhibition of lymphocyte migration caused by fingolimod treatment might carry a similar risk is not known and will require further investigation. These uncertainties notwithstanding, the results of the current proof-of-concept study by Kappos et al. are certainly promising and should provide a strong incentive for long-term follow-up trials on a large scale.

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