the coherence, however, there are numerous tradeoffs in choosing the spectrum, temporal shape, and time delay of the probe pulse to maximize the resonant CARS signal and minimize the contribution of the unwanted background to the signal. Pestov *et al.* find that the frequency spectrum of the CARS signal as a function of probe time delay $t_1 - t_0$ contains information vital for selective detection of the molecule of interest.

The excitation of Raman coherences using femtosecond pump and Stokes pulses is surprisingly efficient, provided that the pulses are Fourier-transform-limited. The frequency linewidths of Q-branch transitions (where the change in rotational quantum number $\Delta J = 0$) in the fundamental vibrational Raman band of N_2 near room temperature and pressure are approximately 0.1 cm^{-1}, a factor of more than 1000 smaller than the 150 cm^{-1} frequency widths of the 100-fs pump and Stokes beams. Despite this drastic difference in frequency widths, there are numerous pump-Stokes frequency pairs underneath the frequency envelopes of the pump and Stokes beams that are separated by the transition frequency of 2330 cm^{-1} (see the figure). If the pump and Stokes beams are Fourier-transform-limited, then these frequency pairs will all contribute to the excitation of Raman coherence with the same phase thus amplifying the signal. Our calculations (*7*) show that for pump and Stokes irradiance levels of 5×10^{17} W/m^2, the Raman coherence is within a factor of two of its maximum possible value (*8*).

Once the Raman coherence is excited by the pump and Stokes beams, the probe beam generates a signal with the desired information. The probe beam can be delayed relative to the pump beams in a controlled manner to monitor the temporal evolution of the CARS signal. This technique has been used to measure, for example, spectroscopic parameters and line-broadening coefficients for nitrogen and other molecules (*9*) and gas temperatures from the frequency-spread dephasing of the macroscopic Raman coherence (*10*).

However, the same information can be obtained by chirping the probe pulse (*11*) and spectrally resolving the resulting CARS signal (*12*); in this case, the temporal decay information is encoded in the spectrum of the CARS signal. Knutsen *et al.* (*2*) used a chirped probe pulse to obtain high spectral resolution in the CARS spectra of organic liquid molecules. Pestov *et al.* use a shaped probe pulse to discriminate against the nonresonant four-wave mixing interference, and spectrally resolved the CARS signal for different probe delays to take advantage of both the temporal and spectral characteristics of the femtosecond CARS signal.

The impressive work of Pestov *et al.* illustrates the continued rapid development of femtosecond CARS techniques. But femtosecond laser systems are by no means limited to coherent Raman processes. A diagram similar to the figure can also be drawn for two-photon absorption resonances such as the 1S-2P transition in atomic hydrogen, which is of fundamental interest in atomic physics. Femtosecond lasers are also widely used for two-

photon-excited fluorescence microscopy (*13*). Meshulach and Silberberg (*14*) used a pulse shaper to demonstrate quantum control of two-photon transitions in cesium. Unlike two-photon absorption experiments with nanosecond laser systems, entire vibrational bands of molecules will be excited when femtosecond-laser systems are used. As with femtosecond CARS, the challenge for researchers will be to take advantage of the characteristics of femtosecond laser radiation to develop new methods for probing these two-photon excited states.

References and Notes

1. C. C. Hayden, D. W. Chandler, *J. Chem. Phys*. **103**, 10465 (1995).
2. K. P. Knutsen, J. C. Johnson, A. E. Miller, P. B. Petersen, R. J. Saykally, *Chem. Phys. Lett*. **387**, 436 (2004).
3. T. Lang, M. Motzkus, H. M. Frey, P. Beaud, *J. Chem. Phys*. **115**, 5418 (2001).
4. J.-X. Cheng, X. S. Xie, *J. Phys. Chem. B* **108**, 827 (2004).
5. D. Pestov *et al*., *Science* **316**, 265 (2007).
6. A. C. Eckbreth, *Laser Diagnostics for Combustion Temperature and Species, Second Edition* (Gordon and Breach, Amsterdam, 1996).
7. S. Roy, R. P. Lucht, T. A. Reichardt, *J. Chem. Phys*. **116**, 571 (2002).
8. M. Jain, H. Xia, G. Y. Yin, A. J. Merriam, S. E. Harris, *Phys. Rev. Lett*. **77**, 4326 (1996).
9. G. Knopp *et al*., *J. Raman Spectrosc*. **33**, 861 (2002).
10. A chirped pulse is lengthened, compared to the Fourier-transform limited pulse, and the wavelength changes systematically during the pulse.
11. R. P. Lucht, S. Roy, T. R. Meyer, J. R. Gord, *Appl. Phys. Lett*. **89**, 251112 (2006).
12. T. Lang, M. Motzkus, *J. Opt. Soc. Am*. B **19**, 340 (2002).
13. W. Denk, J. H. Strickler, W. W. Webb, *Science* **248**, 73 (1990).
14. D. Meshulach, Y. Silberberg, *Nature* **396**, 239 (1998).

IMMUNOLOGY

The Sources of a Lipid Conundrum

Jerold Chun

Distinct sources of a bioactive lipid are needed for lymphocytes to enter the circulation. Therapeutic strategies need to take into account the existence of more than one lipid reservoir.

From diet to waistline, our cultural preoccupation with avoiding fats obscures the fundamental importance of these and related molecules in our lives. Even rare forms of fat have important physiological consequences. A notable example is the lysophospholipids, low-abundance and structurally simple fat molecules derived from cell membranes. Over the last decade, mechanistic understanding of how these lipids are bioactive has revealed their expansive in vivo biology (*1*). On page 295 in this issue, Pappu

et al. (*2*) further develop the story of an immunomodulatory lysophospholipid called sphingosine 1-phosphate (S1P). By investigating the sources of this lipid, a clearer picture of its effects on lymphocyte behavior emerges that may contribute to the development of better immunosuppressive drugs.

The cornerstone for understanding the biological effects of lysophospholipids was discovering that their actions are mediated by cell-surface receptors (*3, 4*). This indicated that lysophospholipids were not lytic (as the prefix "lyso" originally implied), nor were they detergents or constituents of passive membranes, as had been widely assumed based on their structure. Rather, these lipids appeared more like

"keys" that open (activate) receptor "locks." A combination of molecular biology and genetics, on one hand, with lipid biochemistry and pharmacology, on the other, has identified what is now a mammalian family of five S1P and five lysophosphatidic acid (a related lipid) receptors that belong to the well-characterized G protein–coupled receptor superfamily.

Paramount to understanding the in vivo functions of these lipid signals has been the use of "knockout" mice genetically engineered to lack one or more lipid receptors. This approach has proved receptor identity and revealed essential in vivo roles broadly affecting physiology, including the nervous, reproductive, cardiovascular, and immune

The author is at the Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA. E-mail: jchun@scripps.edu

systems. Without cloned receptors and knock-out mice, lysophospholipid biology would likely still be mechanistically ambiguous, just as it was a decade ago when the first receptors were identified (*4*).

Pappu *et al*. have extended this molecular genetic approach to study sphingosine kinase-1 and -2, the enzymes that produce S1P (*5*). Although S1P is predominantly synthesized within cells, it can be released to act as an extracellular signaling molecule. The authors found that genetic removal of both enzymes from hematopoietic, endothelial (vascular and lymphatic), and liver cells in a mouse effectively eliminated S1P circulating in the blood (plasma) and lymph. The resulting physiological consequences, including compensatory

(accompanied by embryonic lethality), thus demonstrating an essential role for these enzymes in normal S1P production (*7*). It also raised the possibility of removing the kinases (and therefore S1P) at a desired age and in specific cell types through genetic approaches. By using a special kind of targeted mutation, whereby expression of both sphingosine kinases could be conditionally prevented in hematopoietic cells and vascular and lymphatic endothelial cells, Pappu *et al*. identified red blood cells as a major sink for S1P. This was further strengthened in mice lacking other blood components (platelets or B and T lymphocytes). These animals retained normal blood concentrations of S1P, consistent with different approaches (*8*) that also identified

relating to how loss of the receptor $S1P_1$ prevents lymphocyte egress. One model, based predominantly on genetic approaches, proposes a direct effect of S1P on $S1P_1$ expressed on lymphocytes (*6*). However, another model, based on the use of chemical compounds, assigns a key role to $S1P_1$ on blood vessels (endothelial cells) where the receptors are proposed to act as "gatekeepers" for lymphocyte egress (*9*). The methodological details of studies supporting each model do not allow a clear resolution of discrepancies. However, two factors support the first model. First, most if not all data derived from genetic mutants of S1P signaling are in concert with the lymphocyte $S1P_1$ model. Pappu *et al*. provide further support through reconstitution experiments in mice in which $S1P_1$ expression is removed from lymphocytes while maintained on the endothelium. This disrupts egress, and indicates that, at the very least, $S1P_1$ on lymphocytes matters. Second, a "home-grown" approach to finding specific chemical compounds that activate (agonists) or block (antagonists) S1P receptors is promising, but at a comparatively preliminary stage compared to highly modified and carefully tested compounds that might someday become a medicine (*10*). Just as proven medicines can have unintended side-effects, so too can preliminary compounds produce results that may be unexpected or at odds with other methodologies.

The therapeutic future of these and other lipid signaling molecules is bright, yet as with many new approaches, unknowns persist. The finding of large reservoirs of S1P in red blood cells presents challenges to strategies proposed for altering blood S1P concentrations as possible therapies [for example, by intravenous delivery of antibodies against S1P into blood (*11*)]. Additionally, based on the dual-source data of Pappu *et al*., altering blood concentrations of S1P may leave local sources of S1P untouched. Other unknowns include as-yet unidentified S1P receptors, interactions with other molecular pathways, clear identification of signaling versus structural lipid pools, and relationships to other lipid pathways, as have been observed for prostaglandins (*12*). A more advanced therapeutic strategy that has entered human trials is the nonselective S1P receptor agonist known as FTY720 or fingolimod, which is being assessed as an immunosuppressive agent for organ transplantation and multiple sclerosis (*13–15*). At least part of the mechanism through which this experimental drug works, benefiting further from the work of Pappu *et al*., is basically conserved between mouse and humans, by virtue of the central role played by S1P receptors. It can be expected that the coming years will bring new
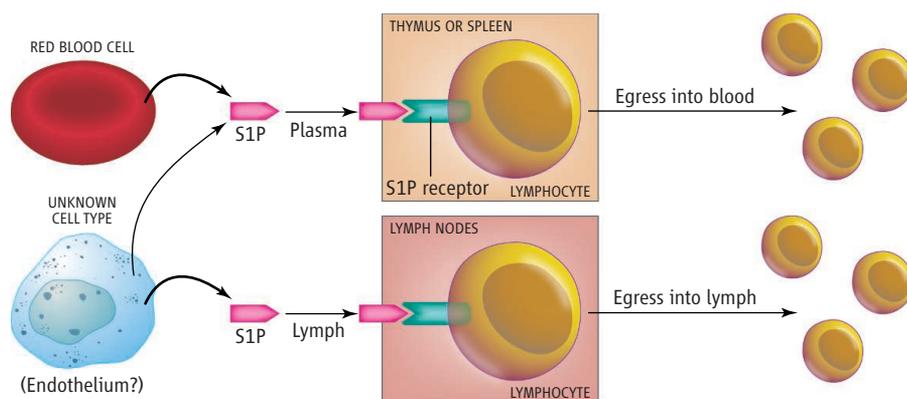
Distinct sources. Sphingosine 1-phosphate (S1P) that is produced in distinct locations acts on receptors ($S1P_1$) expressed on lymphocytes harbored in different organs and tissues. This controls lymphocyte movement into the circulation (blood or lymph).

up-regulation of lymphocyte S1P receptor expression and a major reduction in lymphocyte (T cell, and to a lesser extent, B cell) number in blood and lymph, could be understood based on knowledge of S1P receptor signaling mechanisms in the immune system. In other words, by removing the cell's key-making machinery (sphingosine kinases), the keys are lost and locks stay shut, as expected. This is an important result because it eliminates nonreceptor mechanisms of S1P action in accounting for a well-characterized aspect of in vivo biology—the control of circulating lymphocyte numbers.

During lymphocyte egress, lymphocytes leave their immunological "motels" (lymph nodes, spleen, and thymus) to circulate in the blood and lymph. Previous work demonstrated that an S1P receptor subtype (designated $S1P_1$) is required for this egress because its genetic removal prevents this movement (*6*). Much less clear has been the source of S1P that allows this egress. Previous studies in mice in which both sphingosine kinases were deleted in all cells showed a profound loss of S1P

red blood cells as a source of S1P rather than platelets and thrombocytes, long thought to be major S1P sources. The Pappu *et al*. study also refines the contributions of whole-blood components to concentrations of other lysophospholipids (*4*).

Pappu *et al*. further demonstrate that blood-derived S1P supports the migration of lymphocytes from some locales (spleen and thymus) but not others (lymph nodes). Through clever reconstitution experiments in mice that involved irradiating the animals and replacing their bone marrow (the source of hematopoietic cells), they determined that S1P in the lymph node is from a radiation-insensitive cell source (possibly lymphatic endothelium) that is not in blood. Moreover, this nonblood source also supports lymphocyte migration from the spleen and thymus as well (see the figure). Thus, two distinct sources of S1P—red blood cells and intrinsic radiation-resistant cells present in the lymphocyte "motels"—are needed for normal lymphocyte egress.

Pappu *et al*. address another controversy

mechanistic and therapeutic insights as these "fat" keys open more locks.

References
1. S. E. Gardell, A. E. Dubin, J. Chun, *Trends Mol. Med.* **12**, 65 (2006).
2. R. Pappu *et al.*, *Science* **316**, 295 (2007); published online 15 March 2007 (10.1126/science.1139221).
3. T. Hla, *Prostaglandins Other Lipid Mediat.* **77**, 197 (2005).
4. I. Ishii, N. Fukushima, X. Ye, J. Chun, *Annu. Rev. Biochem.* **73**, 321 (2004).
5. M. Maceyka, S. G. Payne, S. Milstien, S. Spiegel, *Biochim. Biophys. Acta* **1585**, 193 (2002).
6. M. Matloubian *et al.*, *Nature* **427**, 355 (2004).
7. K. Mizugishi *et al.*, *Mol. Cell. Biol.* **25**, 11113 (2005).
8. P. Hanel, P. Andreani, M. H. Graler, *Faseb J.* (2007).
9. H. Rosen, M. G. Sanna, S. M. Cahalan, P. J. Gonzalez-Cabrera, *Trends Immunol.* **28**, 102 (2007).
10. T. Bartfai, G. V. Lees, *Drug Discovery from Bedside to Wall Street* (Elsevier Academic Press, Burlington, MA, 2006).
11. B. Visentin *et al.*, *Cancer Cell* **9**, 225 (2006).
12. X. Ye *et al.*, *Nature* **435**, 104 (2005).
13. V. Brinkmann, J. G. Cyster, T. Hla, *Am. J. Transplant.* **4**, 1019 (2004).
14. L. Kappos *et al.*, *N. Engl. J. Med.* **355**, 1124 (2006).
15. T. Baumruker, A. Billich, V. Brinkmann, *Expert Opin. Investig. Drugs* **16**, 283 (2007).

10.1126/science.1142239

CHEMISTRY

Putting Order into Polymer Networks

Organic, three-dimensional microporous structures have been synthesized. Such "organic zeolites" are light and chemically versatile, offering a range of possible applications.

Peter M. Budd

Microporous materials contain pores or channels with diameters of less than 2 nm—only a little bigger than many molecules. These pores or channels may be used as filters that allow some species through but not others, as containers to isolate or store specific molecules, or as tiny chemical reactors. Chemists have found ways to prepare a wide variety of porous materials, but it has proved difficult to form organic polymer networks with perfectly controlled pore dimensions—until now. On page 268 of this issue, Yaghi and co-workers report the generation of highly porous, organic, three-dimensional crystalline covalent networks (see the figure) (1).

Inorganic microporous materials, such as zeolites, usually have well-defined network structures made up of silicon and aluminum atoms linked via oxygen atoms. Windows and cages within the zeolite framework allow small molecules to access high internal surface areas. Some types of zeolite are found naturally, but many more have been synthesized in the laboratory. In recent years, many researchers have tried to create organic analogs of zeolites. Organic components, although inevitably limited in the temperatures they can withstand, allow much greater control over the chemical nature of the accessible surface. For example, groups may be incorporated that recognize specific molecules or catalyze particular reactions. Furthermore, materials based only on light elements are advantageous in applications where mass must be kept to a minimum, such as for storing hydrogen onboard vehicles.

There is some truth in the saying that nature abhors a vacuum. Thermodynamics works against the formation of tiny spaces and the surfaces that surround them. Zeolites are usually metastable structures and, given appropriate conditions, they will transform to more stable, denser phases. During the preparation of porous materials, the spaces are filled with solvent or other small molecules. All too often, a promising-looking structure disintegrates when the molecules propping up the pores are removed. Many new "porous" materials have been reported, but the porosity is often not permanent in the sense of the material remaining intact on evacuation of the pores.

An important step toward organic zeolites was achieved with the development of materials in which rigid organic components are linked by noncovalent interactions, such as metal-ligand or hydrogen bonds. In particular, crystalline metal-organic frameworks have been produced that exhibit impressive levels of gas uptake (2, 3).

Crystallinity is not necessary for microporosity. Indeed, amorphous porous materi-
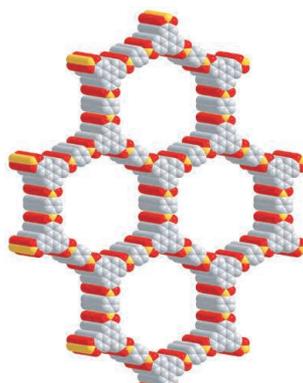
The author is at the Organic Materials Innovation Centre, School of Chemistry, University of Manchester, Manchester M13 9PL, UK. E-mail: peter.budd@manchester.ac.uk
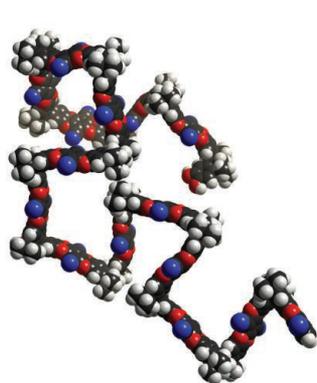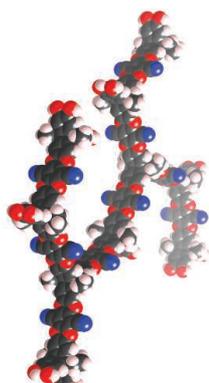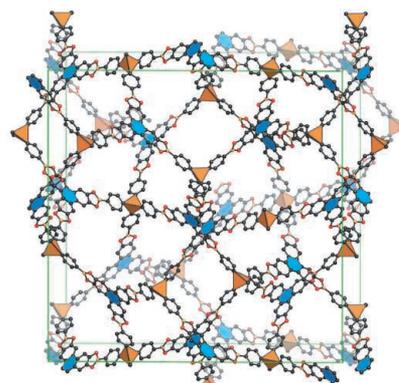
| Membrane-forming microporous polymer | Network-forming microporous polymer | Two-dimensional covalent organic framework | Three-dimensional covalent organic framework |

From disorder to three-dimensional order. Porous polymers can display different degrees of order. From left to right, this figure shows a soluble, membrane-forming microporous polymer (6), a network-forming microporous polymer that exhibits high gas uptake (8), a two-dimensional covalent organic framework (9), and one of the three-dimensional covalent organic frameworks described by Yaghi and co-workers in this issue.