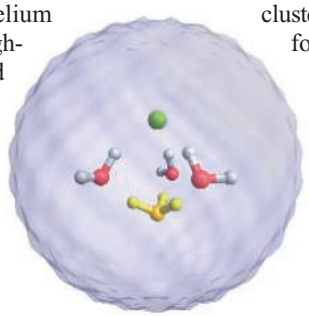
One of the trademarks of helium droplets is their ability to trap high-energy cluster structures behind even small energy barriers to rearrangement (*7*, *8*). The constituents come together in the helium droplet one at a time, steered toward one another by long-range electrostatic interactions during their approach, with excess energy removed quickly by the evaporation of helium atoms from the droplet. In such circumstances, even small energy barriers could prevent ion-pair formation and rearrangement of the hydrogen-bonded network of water molecules. Instead, just the opposite occurs. The calculations uncovered a nearly barrierless pathway to formation of the final separated ion pair $H_3O^+(H_2O)_3Cl^-$ involving addition of a fourth water molecule to cyclic $HCl(H_2O)_3$, facilitated by the step-by-step cluster growth process. The single acceptor–single donor water molecules present in both starting and ending structures have been implicated as required sites for attack by HCl leading to ionization on ice surfaces (*9*, *10*).



Confine and separate. Calculated structure for the $H_3O^+(H_2O)_3Cl^-$ separated ion pair in a helium droplet, as deduced by Gutberlet *et al*.

The remarkable ability of water to stabilize and solvate ions is clearly exposed in this cluster structure. The fact that a mere four water molecules are sufficient to drive proton transfer and to stabilize the ion-pair products testifies to water's unique assets both as a proton acceptor and as a medium for forming strong hydrogen-bonding networks that stabilize the ions once formed.

Much remains to be done in studying acid-base reactions in this small size limit. We still need to gain a deeper understanding of the development of acid-base reactivity as a function of cluster size. In the $HCl(H_2O)_n$ clusters, for instance, we would hope to understand whether the turn-on of ion-pair formation at $n = 4$ is complete, or whether larger cluster sizes might support certain initial structures as neutrals and others as ion pairs. In those that are trapped as one or the other, we would like to study their temperature-dependent stability, or use laser-initiated excitation to drive proton transfer. Additional ways to characterize these clusters are being developed, including measurements of the vibrational transition moment directions (*8*) and the use of double-resonance spectroscopy (*11*) to identify and study individual structural isomers. In the helium droplets, such double-resonance schemes can also be used to measure the barriers to isomerization or to the proton transfer reaction itself (*12*).

The goal ahead will be to squeeze these clusters for every drop of new understanding of acid-base chemistry in the small size limit.

References and Notes

1. W. Lu, C. M. Lieber, *Nat. Mater.* **6**, 841 (2007).
2. R. M. Metzger, *J. Mater. Chem.* **18**, 4364 (2008).
3. W. E. Moerner, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 12596 (2007).
4. A. M. Moore, P. S. Weiss, *Annu. Rev. Anal. Chem.* **1**, 857 (2008).
5. A. Gutberlet *et al*., *Science* **324**, 1545 (2009).
6. J. P. Toennies, A. F. Vilesov, *Angew. Chem. Int. Ed.* **43**, 2622 (2004).
7. C. J. Burnham, S. S. Xantheas, M. A. Miller, B. E. Applegate, R. E. Miller, *J. Chem. Phys.* **117**, 1109 (2002).
8. M. Y. Choi *et al*., *Int. Rev. Phys. Chem.* **25**, 15 (2006).
9. J. P. Devlin, M. Farnik, M. A. Suhm, V. Buch, *J. Phys. Chem. A* **109**, 955 (2005).
10. J. P. Devlin, N. Uras, J. Sadlej, V. Buch, *Nature* **417**, 269 (2002).
11. T. S. Zwier, *J. Phys. Chem. A* **105**, 8827 (2001).
12. G. E. Douberly, J. M. Merritt, R. E. Miller, *J. Phys. Chem. A* **111**, 7282 (2007).

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ECOLOGY

Thriving in Salt

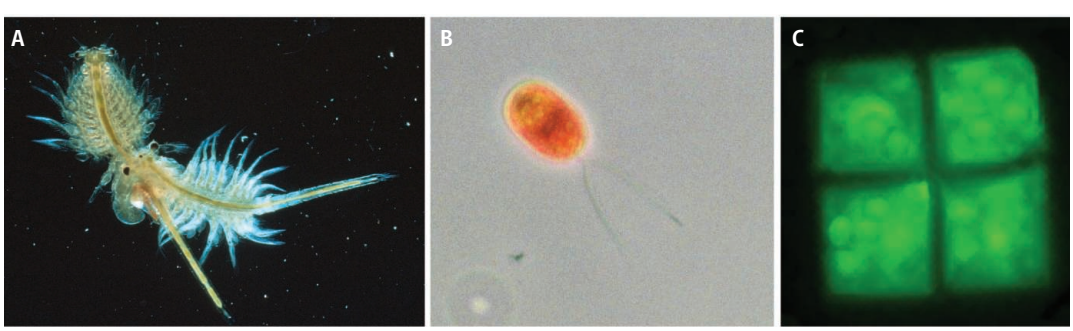
Saline environments from polar glaciers to the deep sea floor contain a surprising variety of organisms.

Antje Boetius[1] and Samantha Joye[2]

Fluids containing ≥5% salt are classified as brines and exclude most life on Earth, but some microbes thrive at salt saturation (35% salt, 10 times the salinity of seawater). Recent findings of novel saline habitats such as subsurface aquifer seeps, deep-sea brine pools, and ancient subglacial brine (*1*–*4*) extend our knowledge on the limits of life on Earth and beyond (*5*) and elucidate how cycling of sulfur, methane, and iron can support microbial ecosystems in chemically isolated habitats in the absence of light (*2*).

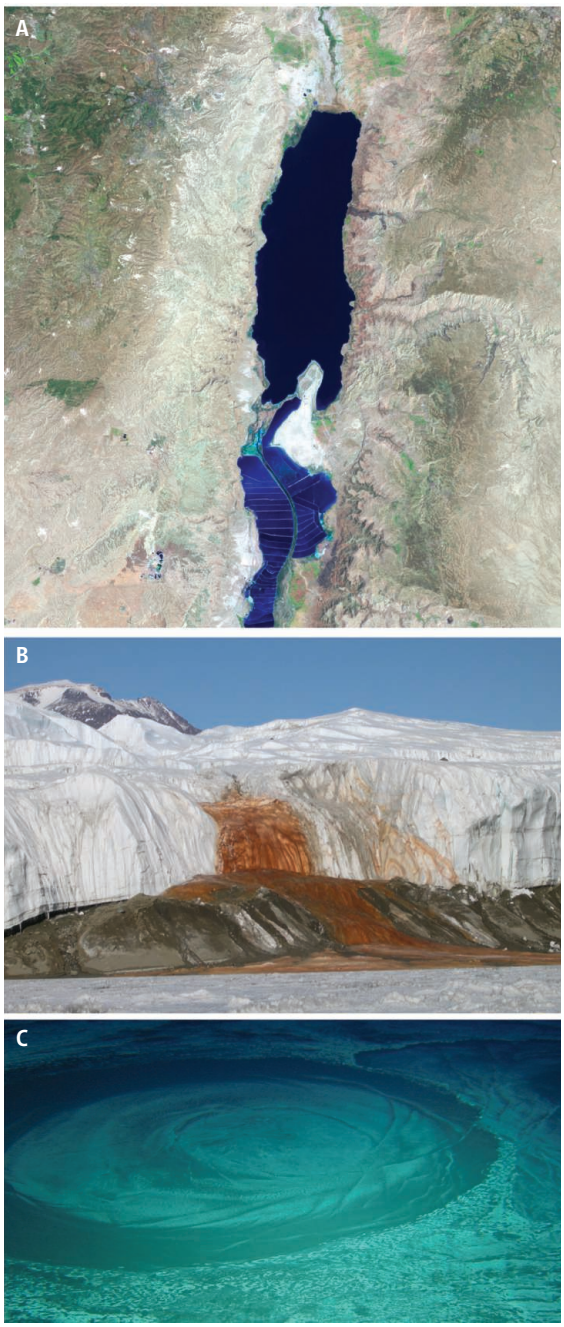
High salt requires microorganisms to minimize water loss and osmotic stress. They accomplish this by concentrating intracellular solutes and by adaptations of their enzymes, which are hence of interest to biotechnology.

The only animal known to tolerate high salt is the "living fossil" brine shrimp *Artemia*, a member of the Branchiopoda. A number of salt lakes have an endemic variety, such as the endangered species *A. monica*, which thrives in Mono Lake, California (see the first figure, panel A). The most famous halophilic algae, *Dunaliella salina*, a very pink member of the Chlorophyceae, survives up to 23% salt (see the first figure, panel B). *Dunaliella* was first



Halophilic life. (**A**) The Mono Lake brine shrimp *Artemia monica* (~1.5 cm long). (**B**) The bright pink phototrophic alga *Dunaliella* (each individual is ~5 μm long). (**C**) A ~5-μm-wide cluster of square archaea *Haloquadratum walsbyi*.

[1]HGF-MPG Joint Research Group for Deep Sea Ecology and Technology, Alfred Wegener Institute for Polar and Marine Research, 27515 Bremerhaven, Germany, and Max Planck Institute for Marine Microbiology, 28359 Bremen, Germany. [2]Department of Marine Sciences, University of Georgia, Athens, GA 30602, USA. E-mail: aboetius@mpi-bremen.de; mjoye@uga.edu



Brine habitats. (**A**) The Dead Sea, where the first halophilic organisms were identified. (**B**) Blood Falls at the base of the Taylor Glacier in Antarctica. (**C**) A bacterial mat–covered brine pool on a deep-sea mud volcano in the Eastern Mediterranean.

studied by Baas-Becking in 1931 (*6*) and was the first life form documented in the Dead Sea (*7*, *8*). The extremely halophilic archaeon *Haloquadratum walsbyi*, first found in a Red Sea salt pond (*9*), has been cultivated and its genome sequenced (*10*). It is <0.1 μm thick and has an unusual rectangular shape (see the first figure, panel C). This and other halophilic archaeae form gas vesicles to float in the sun-lit, aerobic, nutrient-rich brines.

Halophilic organisms are found in many branches of the tree of life, and salt-adapted enzymes occur in both archaea and bacteria. These observations suggest either that similar adaptation mechanisms arose numerous times or that lateral gene transfer is an important mechanism for halophile evolution. Either mechanism can account for the widespread distribution of carotenoid and rhodopsin pigments in halophilic bacteria and archaea. This pigmentation explains the red color of salt lakes, salterns, and salt flats.

Various mechanisms generate the diverse hypersaline habitats found on Earth. Salt lakes such as the giant Aral Sea or Lake Balkhash represent a substantial fraction of inland water bodies. They form when geological processes create a basin in which water evaporation exceeds water input. Evaporation is also used to precipitate salt commercially in human-made shallow salt ponds (salterns) retaining water from the sea or from salt lakes. Salt has been a valuable commodity since the Bronze age, and many ancient cities gained wealth from mining, making, and marketing salt.

Historically, the most intriguing salt lake is the Dead Sea (see the second figure, panel A), mentioned in the Book of Genesis and in Pliny the Elder's *Natural History* as being free of life. The tectonic isolation of the Dead Sea basin from the ocean has contributed to its slow salinization. Today, this natural laboratory is endangered because of ever-decreasing freshwater inputs from the Jordan River. Volcani first described microbial life in Dead Sea waters in his benchmark 1936 paper (*8*). Many halophilic archaeae were isolated from samples of Volcani 57 years after their collection (*11*), indicating low mortality in their salty habitat. For the same reason, thick photosynthetic microbial mats can thrive at the bottom of shallow salt lakes where their grazers are excluded.

Brines also occur in polar habitats. When sea ice forms, 10 to 30% of its volume is comprised by brine channels. Sea-ice microbial communities thrive inside the interconnected brine channels that concentrate nutrients and organic substrates (*12*). In Antarctica, subglacial saline lakes and surficial brine lakes or moats are common (*1*, *13*). The Antarctic Dry Valleys are home to one of the most intriguing and spectacular microbial ecosystems known (*2*): Blood Falls, where subglacial iron-rich seawater-derived brine episodically drains from Taylor Glacier (see the second figure, panel B). This brine contains a low diversity of microbes, mainly sulfur- and iron-using bacteria, including the Arctic species *Thiomicrospira arctica* (*14*).

Finally, deep, dark, and remote hypersaline habitats—such as mud volcanoes, brine lakes, and anoxic basins—occur at the bottom of the Black, Red, and Mediterranean seas and in the Gulf of Mexico (see the second figure, panel C). When subsurface fluids interact with ancient salt deposits, they can form warm brines, which migrate up through the sediments and are expelled at the sea floor or accumulate in brine pools. The microbial ecosystems associated with these brines vary with fluid composition and flow rate (*3*). Hypersaline anoxic basins in the eastern Mediterranean Sea result from the dissolution of Miocene salt deposits exposed to seawater after tectonic activity (*15*). Brine microbial ecosystems are associated with mud volcanoes along the tectonically active Mediterranean Ridge and on the passive Egyptian continental margin, where subsurface fluids interact with Messinian salt deposits as a result of gravitational transport (*16*).

The high density of hypersaline brine limits mixing with overlying seawater, generating a chemocline up to a few meters thick (*3*). Viewing such habitats from a submersible is like seeing a lake in the ocean (see the second figure, panel C). These chemoclines host an elevated biomass and diversity of microorganisms relative to the overlying seawater and underlying brine, including novel bacterial and archaeal clades (*17*). Microorganisms profiting from the redox gradients, such as sulfide-oxidizing *Arcobacter*, leave visible sulfur precipitates floating on the brine (*16*). In Gulf of Mexico brines, which contain no sulfate, specific microbial functions—such as formation and consumption of acetate and subsequent methane production—have been observed for the first time at high salinity and were found to vary across the chemocline (*3*).

Saline habitats and their diverse halophilic inhabitants will remain an important focus for extremophile studies, furthering our knowledge of their unique adaptations and providing novel enzymes for biotechnological applications. In addition, remote and isolated briny

habitats such as those described in recent studies (*1*–*4*) are fascinating natural laboratories to study the persistence, energetics, and complexity of microbial life, with implications for the evolution of biogeochemical cycles on Earth and elsewhere.

References and Notes

1. W. J. Green, W. B. Lyons, *Aquat. Geochem.* **15**, 321 (2009).
2. J. A. Mikucki *et al.*, *Science* **324**, 397 (2009).
3. S. B. Joye *et al.*, *Nat. Geosci.* **2**, 349 (2009).
4. B. A. Biddanda *et al.*, *Eos* **90** (8), 61 (2009).
5. R. L. Mancinelli, T. F. Fahlen, R. Landheim, M. R. Klovstad, *Adv. Space Res.* **33**, 1244 (2004).
6. L. G. M. Baas-Becking, *J. Gen. Physiol.* **14**, 765 (1931).
7. A. Oren, *Saline Syst.* **4**, 2 (2008).
8. B. Wilkansky, *Nature* **138**, 467 (1936).
9. A. E. Walsby, *Nature* **283**, 69 (1980).
10. H. Bolhuis *et al.*, *BMC Genomics* **7**, 169 (2006).
11. D. R. Arahal *et al.*, *Appl. Environ. Microbiol.* **62**, 3779 (1996).
12. J. A. Mikucki, J. C. Priscu, *Appl. Environ. Microbiol.* **72**, 4029 (2007).
13. D. N. Thomas, G. S. Dieckmann, *Science* **295**, 641 (2002).
14. M. J. Siegert *et al.*, *Nature* **414**, 603 (2001).
15. P. W. J. J. van der Wielen *et al.*, *Science* **307**, 121 (2005).
16. E. O. Omoregie *et al.*, *Appl. Environ. Microbiol.* **74**, 3198 (2008).
17. W. Eder *et al.*, *Environ. Microbiol.* **4**, 758 (2002).

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IMMUNOLOGY

A Chronic Need for IL-21

A cytokine produced by helper T cells enables CD8$^+$ T cells to control viral infection.

Lisa D. S. Johnson and Stephen C. Jameson

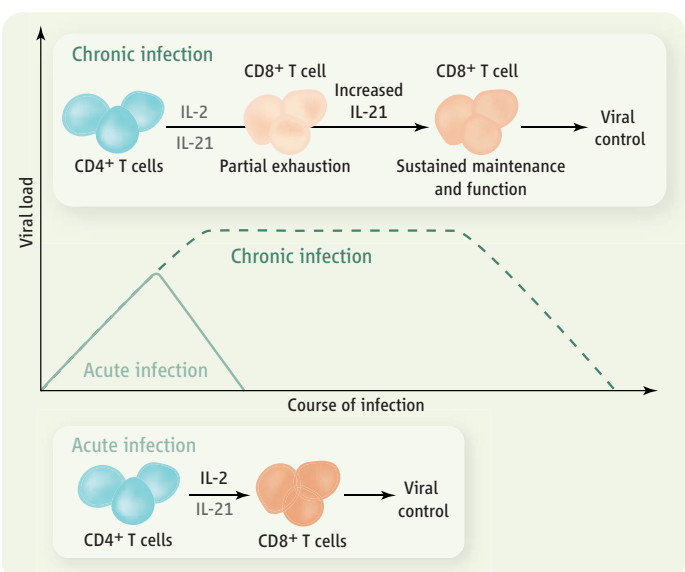
Chronic viral infections provoke a war of attrition between cytotoxic T cells (the CD8$^+$ subclass) of the immune system and infected host cells. Although the CD8$^+$ T cell response gradually whittles away at the viral load, the cells progressively become "exhausted" and lose function during a chronic infection (*1*). This exhaustion becomes more severe in the absence of helper CD4$^+$ T cells, potentially leading to viral persistence and loss of virus-specific CD8$^+$ T cells (*1*, *2*). But how do CD4$^+$ cells help? Three reports in this issue, by Elsaesser *et al.* on page 1569 (*3*), Yi *et al.* on page 1572 (*4*), and Fröhlich *et al.* on page 1576 (*5*), suggest an unexpected role for a specific cytokine produced by CD4$^+$ T cells called interleukin-21 (IL-21) in controlling chronic viral infections.

IL-21 is produced by multiple CD4$^+$ T cell subsets, prominently T helper 17 (T_H17) cells, follicular helper T cells, and natural killer T cells (*6*, *7*). The cytokine enhances differentiation of some of these subsets, but other immune cells, including B, natural killer, and CD8$^+$ T cells, also respond to IL-21. The IL-21 receptor (IL-21R) belongs to the γC family of cytokine receptors (which includes receptors for IL-2, -7 and -15), and IL-21 can cooperate with other members of that group to enhance CD8$^+$ T cell responses (*8*, *9*). This lays the groundwork for considering whether CD4$^+$ cell "help" for CD8$^+$ T cell responses involves IL-21 as an intermediary.

The new reports show that in mice, IL-21 is produced by CD4$^+$ T cells responding to chronic lymphocytic choriomeningitis virus (LCMV) infection. As the infection persists, these T cells increase IL-21 production (while decreasing IL-2 production) (*3*, *4*) (see the figure). To test the relevance of this response, IL-21– or IL-21R–deficient mice were chronically infected with LCMV. Surprisingly, the studies found that both types of mice were more susceptible than normal mice to chronic infection, and had more dramatic exhaustion of LCMV-specific CD8$^+$ T cells. Furthermore, IL-21– and IL-21R–deficient mice could not overcome chronic infection compared to normal mice that were infected. By contrast, the magnitude of the CD4$^+$ T cell response was normal [or even enhanced (*3*)] in the IL-21– and IL-21R–deficient mice. Antibody production by B cells to LCMV was mildly impaired in IL-21– and IL-21R–deficient animals (IL-21 simulates B cell function) (*6*, *7*), but antibody is not thought critical for controlling chronic LCMV infection.

Because the sustained presence of viral antigen during chronic infection reinforces CD8$^+$ T cell exhaustion, IL-21 might enhance viral clearance by affecting other cells types (e.g., CD4$^+$ T cells). This might also relieve CD8$^+$ T cell exhaustion. The new studies addressed this issue by assessing the function of IL-21R–deficient CD8$^+$ T cells in normal recipient mice. CD8$^+$ T cells lacking IL-21R had impaired self-maintenance, suggesting an autonomous requirement for IL-21 sensitivity. Whether IL-21 also acts on other immune cell types to resolve chronic LCMV infection remains to be determined.

But is IL-21 the critical element that allows CD4$^+$ T cells to help CD8$^+$ T cells combat chronic viral infection? Yi *et al.* report that in response to injected IL-21, CD4$^+$-deficient mice with a chronic LCMV infection showed enhanced numbers and function of CD8$^+$ T cells and reduced viral titers. Although none of the new studies conclusively show that IL-21 produced by CD4$^+$ T cells is essential for helping CD8$^+$ T cells during chronic LCMV infection, the prominence of CD4$^+$ T cells as a source for IL-21 is highly suggestive. IL-21 shows promise in



Help for the exhausted. During acute viral infection, IL-21 is produced by antigen-specific CD4$^+$ T cells, but is not required for the specific CD8$^+$ T cell response or control of the pathogen (solid curve). During chronic viral infection (dashed curve), the CD4$^+$ and CD8$^+$ T cell responses become partially "exhausted," but CD4$^+$ T cells eventually increase IL-21 production. This helps the CD8$^+$ T cell response, eventually leading to viral control.

CREDIT: N. KEVITIYAGALA/*SCIENCE*

Laboratory Medicine and Pathology, Center for Immunology, University of Minnesota, Minneapolis, MN 55455, USA. E-mail: james024@umn.edu

Thriving in Salt

Antje Boetius and Samantha Joye

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