despite their capacity to secrete IL-4 in response to antibody to CD3. These results thus leave open the question of whether early cytokine production by NK1.1+ T cells, by itself, is sufficient to cause Ig class switching or whether conventional CD4+ T cells are important for such help. The earlier work of Goroff et al. (20), indicating that monoclonal C57BL/6 antibodies to BALB/c IgD can elicit IgG1 and IgE responses in BALB/c mice but not in (BALB/c × C57BL/6) F1 mice, strongly suggests that conventional T cells capable of recognizing peptides derived from anti-IgD of C57BL/6 origin are generally required for these responses.

The specificity of NK1.1+ T cells for CD1 (4) and the demonstration that cells genetically capable of expressing CD1 are essential for restoring the capacity of β, M-/- mice to produce IgE indicate that recognition of CD1 may be essential for activating this pathway of priming for IL-4 production. In mice, CD1 is known to be expressed by cortical thymocytes (21) and on gastrointestinal epithelium (22). In humans, CD1 is also expressed on epidermal Langerhan's cells (23), and a distinct CD1 isoform is expressed on a subpopulation of B cells (24). It is possible that the stimuli that elicit IgE production, possibly including infection with helminthic parasites and exposure to various allergens, occur either at sites of constitutive CD1 expression, such as the gastrointestinal tract and the skin, or in response to stimuli that cause increases in peripheral expression of CD1. Such CD1 expression could activate IL-4 production by CD4+ NK1.1+ T cells, or possibly by a population of γδ T cells (25), at the same time as antigen-specific precursors of Th1 cells encounter their complementary ligands. This would provide the IL-4 essential for the priming of such precursor cells to develop into IL-4—producing Th2 cells and for the development of the type of antibody-dominated immune responses that are characterized by high levels of IgE.

13. Fluorescence staining was performed at 4°C in 100 μl containing 10⁶ spleen cells and fluorescein isothiocyanate–conjugated antibody to mouse CD4 (RM4-5; Pharmingen, San Diego, CA) combined with phycoerythrin-conjugated antibody to mouse NK1.1 (PK136; Pharmingen) in phosphate-buffered saline containing 0.1% fetal bovine serum and 0.5% NaNO₃. Fluorescence analysis was done on a FACScan flow cytometer (Becton Dickinson). For acute cytokine production, we removed spleen cells 90 min after injection of antibody to CD3 (2C11; 1.33 μg per mouse). Cell suspensions were washed twice with Hanks' balanced solution. Spleen cells (5 × 10⁶ per well) were cultured in 24-well plates without additional stimulus for 1 h. Supernatants were harvested to measure IL-4, IL-2, and IFN-γ content. CT-4S, an IL-4–dependent cell line, and CT-ET, an IL-2–dependent cell line, were used to measure IL-4 and IL-2 content, respectively. We compared responses from serial dilutions of the supernatants with those detected by ELISA using mouse recombinant IL-2 (Cetus Corp., Emeryville, CA). One unit of IL-4 is equal to 0.5 pg; 1 U of IL-2, defined as the unit, is equal to 0.3 ng/ml. Interferon-γ was assayed with a specific two-site enzyme-linked immunosorbent assay (ELISA) with known amounts of recombinant IFN-γ (Genzyme, Cambridge, MA) as standards. To induce IgE production, we injected mice intravenously with 200 μg of purified goat antibody to mouse IgG plus 600 μg of normal goat Ig. Sera were obtained 8 days later, and serum IgE content was measured with an ELISA. Homocytogous knockout mice were derived from animals in their fourth to eighth generation of backcrosses to C57BL/6 mice.
14. T. Yoshimoto et al., data not shown.
18. F. D. Finkelstein et al., ibid. 151, 1235 (1993).
19. CD8– HSA+ thymocytes were prepared by one-step killing at 37°C with monoclonal antibodies to HSA (J11D2) and CD8 (5.155) plus a low-toxicity rabbit complement (Cedarlane Laboratories, Hornby, Ontario, Canada), followed by centrifugation over a density gradient. To enrich the NK1.1+ CD44+ thymocytes, we removed 3G11+ and lymphocyte endothelial cell adherence molecule-1+ cells from the CD8– HSA+ C57BL/6 thymocyte population with a mixture of biotinylated monoclonal 3G11 and MEL-14 with streptavidin-coated paramagnetic beads (Miltenyi Biotec, Bergish Gladbach, Germany). Cells were separated with a magnetic cell separation system.

The structure of human interleukin-1β (hIL-1β) has a nonpolymer “hydrophobic” cavity that, in three independent crystal structures (1), appears to be empty. On the basis of nuclear magnetic resonance (NMR) data, however, J. A. Ernst et al. (2) suggest that the cavity contains disordered solvents. They identify protons on the protein that display nuclear Overhauser enhancement (NOE) cross-peaks with water molecules that are purportedly within the cavity. We question, first, whether the water molecules that display NOEs are in fact in the cavity, and second, whether appropriate controls are available showing that NOEs are not shown to protons that are remote from the cavity and from solvent-exchangeable sites. The protons identified by Ernst et al. (2) are within side chains near the cavity and include the methyl protons of Leu⁰, Leu⁴, Leu⁶, Leu⁸, Leu⁰, Leu⁴, Ile⁰, Leu⁰, and Val⁰. Some of the β-methylene protons of Leu⁰ and Leu⁸, the γ-methylene protons of Leu⁰ and Leu⁸, and the β-methylene and γ-methylene protons of Ile⁰ suggest that the cavity is very hydrophobic. The NMR experiment does not provide the actual location of the water proton, only that it is relatively close to the protein proton [stated by Ernst et al. (2) to be less than about 5 Å]. We examined the structure of hIL-1β, as determined by NMR (3), to investigate the environments of the protons listed above.

**REFERENCES AND NOTES**


**TECHNICAL COMMENTS**

Use of NMR to Detect Water Within Nonpolar Protein Cavities

The structure of human interleukin-1β (hIL-1β) has a nonpolymer “hydrophobic” cavity that, in three independent crystal structures (1), appears to be empty. On the basis of nuclear magnetic resonance (NMR) data, however, J. A. Ernst et al. (2) suggest that the cavity contains disordered solvent. They identify protons on the protein that display nuclear Overhauser enhancement (NOE) cross-peaks with water molecules that are purportedly within the cavity. We question, first, whether the water molecules that display NOEs are in fact in the cavity, and second, whether appropriate controls are available showing that NOEs are not shown to protons that are remote from the cavity and from solvent-exchangeable sites. The protons identified by Ernst et al. (2) are within side chains near the cavity and include the methyl protons of Leu⁰, Leu⁴, Leu⁶, Leu⁸, Leu⁰, Leu⁴, Ile⁰, Leu⁰, and Val⁰. Some of the β-methylene protons of Leu⁰ and Leu⁸, the γ-methylene protons of Leu⁰ and Leu⁸, and the β-methylene and γ-methylene protons of Ile⁰ suggest that the cavity is very hydrophobic. The NMR experiment does not provide the actual location of the water proton, only that it is relatively close to the protein proton [stated by Ernst et al. (2) to be less than about 5 Å]. We examined the structure of hIL-1β, as determined by NMR (3), to investigate the environments of the protons listed above.
There are, in principle, 26 distinguishable sets of protons. In 18 of these 26 cases we found that at least one proton from each set had a water proton within 5 Å. In four additional cases a water proton was within 6 Å. These water protons are on solvent molecules that form hydrogen bonds to backbone amide and carboxyl groups (1) and are of the sort often seen in x-ray crystal structures of proteins. Bearing in mind that the distance calculation included only the seven water molecules identified in the initial NMR analysis (3), and that both the x-ray studies (1) and the recent NMR analysis (2) identify additional bound solvent molecules that were not included, it appears to us that the majority of the large distance cross-peaks attributed by Ernst et al. (2) to solvent molecules within the hydrophobic cavity might be a result of water molecules bound elsewhere in the protein.

As a control to the NMR experiment, one would expect that methyl protons that are more than 5 Å from bound solvent, from exchangeable protons, and from the cavity should not display NOEs to water. An example of this sort is provided by Val58, although this is not discussed by Ernst et al. (2). On the basis of the refined coordinates of Priestle and Grütter (1), the two methyl groups of Val58 are 5.1 Å and 7.0 Å from the nearest crystallographically observed water molecules. These are carbon-oxygen distances. Because the alignment of the water molecule is unknown, the corresponding proton-proton distances could be somewhat longer or somewhat shorter. (The shortest proton-proton distances to the nearest NMR-identified water are 5.8 Å and 9.0 Å.) The methyl groups of Val58 are also approximately 10 Å and 8 Å, respectively, from the closest possible waters in the nonpolar cavity and 5.1 Å and 7.2 Å from bulk water at the protein surface. Notwithstanding these apparently long distances, especially for Cδ, the methyl protons on Cβ and Cγ of Val58 are identified in figure 1A of the report by Ernst et al. (2) as being involved in direct NOEs with water. The strong NOEs between these protons and water suggest either that distances larger than 5 Å can produce sizeable NOEs or that water molecules have access to other regions of the structure than those suggested by both the crystal and solution structures.

As a further check we calculated the expected oxygen-carbon distances from putative water molecules in the cavity to the side chains that are suggested by Ernst et al. (2) to make NOEs to cavity waters. The closest approaches that a water molecule of radius 1.4 Å within the cavity of hIL-1β can make to the Cα atoms of Leu10, Leu15, Leu60, and Leu89, respectively, are 5.3 Å, 5.7 Å, 5.3 Å and 6.0 Å, that is, in all cases in excess of 5 Å. These distances are in the same range as seen for the crystallographically observed water molecules described above. Whether the NOEs are made over such long distances, or the NOEs are made to waters that penetrate the entire structure (as compared with Val58 above) is an important question with implications both for protein dynamics and energetics. It is to be hoped that further experiments will shed light on these questions. In any event, it remains to be proven that the water molecules that display NOEs with the protons in hIL-1β are necessarily in the hydrophobic cavity.

We do not wish to suggest that nonpolar cavities within proteins are entirely devoid of solvent. The dynamic behavior of proteins allows ready access of nonpolar ligands to internal cavities (4) and water molecules to internal sites (5). In crystal structures of proteins, nonpolar cavities rarely display electron density that can be interpreted as bound solvent (6, 7). This includes cavities that are large enough to accommodate a water molecule, but sufficiently small to restrict the motion of the water molecule such that it should be readily detectable (7, 8). The widespread crystallographic observation that nonpolar cavities of this size do not contain significant electron density provides strong evidence that the occupancy of these cavities by water is, in fact, low.

Brian W. Matthews  
Department of Physics and  
Howard Hughes Medical Institute,  
Institute of Molecular Biology,  
University of Oregon,  
Eugene, OR 97403, USA  
Andrew G. Morton  
Department of Chemistry, and  
Howard Hughes Medical Institute,  
Institute of Molecular Biology,  
University of Oregon  
Frederick W. Dahlquist  
Department of Chemistry,  
Institute of Molecular Biology,  
University of Oregon

REFERENCES


15 May 1995; accepted 27 June 1995
Use of NMR to Detect Water Within Nonpolar Protein Cavities
Science 270 (5243), 1847-1849. [doi: 10.1126/science.270.5243.1847]

Editor's Summary

This copy is for your personal, non-commercial use only.

**Article Tools**
Visit the online version of this article to access the personalization and article tools:
http://science.sciencemag.org/content/270/5243/1847

**Permissions**
Obtain information about reproducing this article:
http://www.sciencemag.org/about/permissions.dtl