ICl and eject an I atom back to the gas phase (Cl-selective abstraction) despite the much larger exothermicity for the formation of Si–Cl(s) + I than Si–I(s) + Cl.

The reorientation by the surface would also explain the higher abstraction ratio for ICl over that for Br_2. An end-on geometry has been calculated to favor abstraction over dissociation for halogens on semiconductor and metal surfaces because this geometry places the terminal halogen far from the surface in an optimal position for abstraction (*2*, *11*). Although molecules in the molecular beam usually have a nearly side-on or tilted orientation, reorientation by the surface should place a large fraction of the ICl into an end-on geometry and thus increase abstraction.

We propose that the orientation of ICl into I-end–first configuration results from the higher polarizability of I compared to Cl and the asymmetric molecular bonding associated with ICl. Because Cl is much more electronegative than I, the bonding σ_z and $\pi_{x,y}$ orbitals predominantly consist of Cl 3*p* orbitals, whereas the antibonding σ_z* and $\pi_{x,y}$* orbitals predominantly consist of I 5*p* orbitals. The highest occupied molecular orbital (HOMO) is mainly concentrated at the I atom of an ICl molecule; thus, the I end is both more polarizable and more reactive than the Cl end. This difference was confirmed by Hartree-Fock molecular orbital calculations made using the SpartanPlus program (*12*) which show that the effective radius of the π*-orbital wave function is 70% greater on the I atom than on the Cl atom. Conversely, there is only 10% difference in the effective radius of the probability density of the entire valence shell between the I and the Cl atoms. Therefore, we suggest that when an ICl molecule approaches a Si adatom on the Si(111)-(7×7) surface, the interaction of an ICl $\pi_{x,y}$* antibond (HOMO) with the partially filled Si dangling bond results in greater attraction to the I end than the Cl end of the ICl molecule. This selection is the driving force for the I-end–first orientation of ICl before reaction and ultimately causes chemical selectivity for the reaction of ICl with the Si(111)-(7×7) surface. This same molecular-orbital argument was used to explain the chemical selectivity of the D + ICl → DI + Cl gas-phase reaction (*1*). Reorientation of NO (*13*) by Ag(111) or Pt(111) and of H_2 by W(100) (*14*) or Pd(100) (*15*) is denoted as rotational steering and has been observed in theoretical simulations. Therefore, the reorientation of molecules by surfaces may be a general phenomenon and is probably the dynamic mechanism responsible for this example of atomically selective chemisorption, the selective abstraction of I from ICl by Si(111)-(7×7).

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Differential Effects of Cytolytic T Cell Subsets on Intracellular Infection

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In analyzing mechanisms of protection against intracellular infections, a series of human CD1-restricted T cell lines of two distinct phenotypes were derived. Both $CD4^-CD8^-$ (double-negative) T cells and $CD8^+$ T cells efficiently lysed macrophages infected with *Mycobacterium tuberculosis*. The cytotoxicity of $CD4^-CD8^-$ T cells was mediated by Fas-FasL interaction and had no effect on the viability of the mycobacteria. The $CD8^+$ T cells lysed infected macrophages by a Fas-independent, granule-dependent mechanism that resulted in killing of bacteria. These data indicate that two phenotypically distinct subsets of human cytolytic T lymphocytes use different mechanisms to kill infected cells and contribute in different ways to host defense against intracellular infection.

Effective immunity to intracellular bacterial infection often requires the lysis of infected cells as well as killing of the invading pathogen. A possible role for cytolytic T lymphocytes (CTLs) in protection against *M. tuberculosis* has been suggested by experiments in mice bearing a disruption in the β_2-microglobulin gene. These mice are unable to express major histocompatibility complex (MHC) class I or class I–like molecules or to generate CTLs and were shown to be highly susceptible to infection (*1*). Despite numerous studies of $CD4^+$ T cell responses and cytokine production in tuberculosis, there remain only a few reports of $CD8^+$ CTLs that recognize mycobacterial antigens (*2*). This paradox led us to investigate whether other antigen-presenting systems could be essential for generation of *M. tuberculosis*–specific CTLs. CD1 is an MHC-like surface molecule with a unique ability to process and present nonpeptide antigens to T cells, including mycobacterial lipids (*3*, *4*). We examined whether CD1-restricted CTLs have the capacity to recognize and lyse *M. tuberculosis*–infected macrophages.

CD1-restricted T cells were derived from patients with active tuberculosis as well as healthy donors (*5*). All of these T cell lines recognized *M. tuberculosis* lipid and lipoglycan antigens in a CD1b-restricted manner as assessed by antigen-

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specific T cell proliferation and interferon-γ (IFN-γ) secretion. T cells were either $CD4^-CD8^-$ (double negative, DN) or $CD8^+$ and expressed αβ T cell receptors, consistent with our previous findings (*3*, *4*, *6*). We investigated whether CD1-restricted T cells recognized antigen-presenting cells (APCs) harboring live mycobacteria. $CD1^+$ macrophages were infected with virulent *M. tuberculosis* with 90% efficiency, such that there were approximately three bacteria per macrophage (*7*). All DN and $CD8^+$ T cell lines examined efficiently lysed infected macrophages in a dose-dependent manner (Fig. 1A). The restriction and specificity were shown by the inhibition of CTL-mediated lysis of infected targets and release of IFN-γ by antibody to CD1b (Fig. 1B). CTLs did not lyse uninfected $CD1^+$ macrophages.

CTLs lyse targets by two pathways, the exocytosis of granules containing perforin and granzymes and the interaction of Fas ligand on the CTL with Fas on the target cell (*8*). The mechanisms operate independently: For example, mice with a disrupted perforin gene retain the ability to exert Fas-FasL–dependent T cell lysis, but the biological roles and contribution to immunity of each remains unresolved. Because *M. tuberculosis*–infected macrophages were killed by two phenotypic subsets of cytotoxic T cells, we sought to clarify the mechanisms of lysis, specifically the relative importance of killing by Fas-FasL interaction and by the degranulation mechanism. The cytotoxicity mediated by two DN, CD1-restricted CTL lines was markedly inhibited by blocking antibodies to Fas or to FasL (Fig. 2A). In contrast, the cytotoxicity of two $CD8^+$ CD1-restricted CTL lines was not affected by blocking of Fas or FasL (Fig. 2B). We also determined the contribution of the granule-dependent pathway to the target cell lysis. Strontium ions (Sr^{2+}), which release histamine from mast cells by inducing granular degranulation, also induce degranulation of cytotoxic lymphocytes (*9*), thereby transiently inhibiting lytic activity. This effect was used to determine the extent to which the granule-dependent pathway participated in killing *M. tuberculosis*–infected macrophages (*10*). Preincubation with Sr^{2+} selectively inhibited the cytotoxicity of the $CD8^+$, but not DN, CD1-restricted CTLs (Fig. 2, A and B). Granzyme A, characteristic of cytotoxic granules, was detected in Sr^{2+}-induced supernatants of $CD8^+$, but not DN, T cells (Fig. 2C). The capacity of lymphocytes to proliferate and release IFN-γ upon antigen-specific activation was not affected by treatment with Sr^{2+} (*11*). The differential ability of antibodies to Fas-FasL or of Sr^{2+} to inhibit CTL activity was not dependent on the level of killing (Fig. 2, A and B).

A critical component of lymphocyte cytotoxic granules is perforin, which polymerizes on the target cell membrane after antigen activation and induces a nonselective pore that may be responsible for target cell lysis (*12*). Using reverse transcriptase–polymerase chain reaction (RT-PCR), we detected induction of perforin mRNA in all three $CD8^+$ CTL lines examined, but not in three DN CTL lines (Fig. 2D). In contrast, mRNA for FasL was detected in stimulated DN CTL lines but not in the $CD8^+$ lines.

The existence of two populations of human CTLs, differentiated by phenotype and by mechanism of cytotoxicity, was confirmed in a larger group of CTLs. Five DN CTL lines independently derived from different donors, all CD1-restricted, killed targets by the Fas-FasL pathway, with little contribution from the granule-dependent mechanism (Fig. 3). Conversely, the cytotoxicity of three $CD8^+$ CD1-restricted CTL lines was granule-dependent. In addition, the killing by two classical $CD8^+$ MHC class I–restricted CTL lines specific for influenza peptide was almost

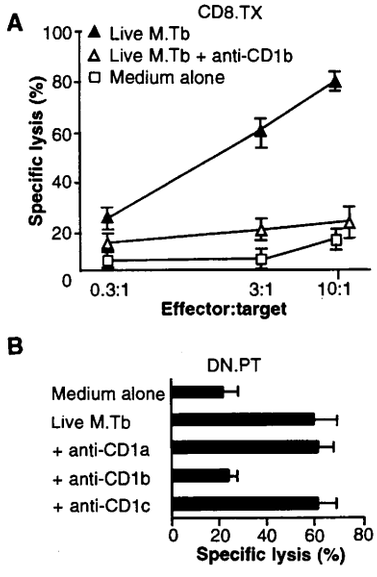

Fig. 1. Cytotoxicity of CD1-restricted CTLs against macrophages infected with virulent *M. tuberculosis*. The cytotoxic response of (**A**) $CD8^+$ line (CD8.TX) and (**B**) DN line (DN.PT, E:T = 10:1) against infected macrophages was measured in a ^{51}Cr-release assay in the presence or absence of blocking antibodies to CD1 (anti-CD1) (*24*). The results shown are representative of one out of three independent experiments, each performed in triplicate. Error bars correspond to the SEM.

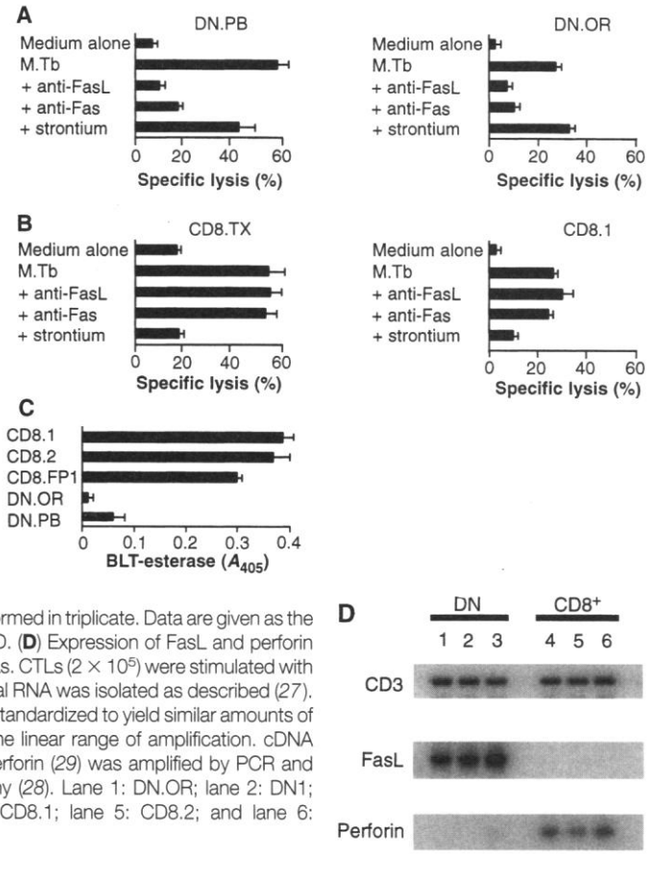

Fig. 2. Distinct mechanisms of cytotoxicity of DN and $CD8^+$ CTLs. Cytotoxicity of (**A**) DN (DN.PB and DN.OR) or (**B**) $CD8^+$ (CD8.TX and CD8.1) CTLs against antigen-pulsed macrophages was determined in the presence or absence of blocking antibodies to FasL (5 μg/ml) or Fas (1 μg/ml) or after initial treatment of the CTLs with Sr^{2+} (*25*). The E:T ratio was 10:1. The result shown is representative of three independent experiments. Error bars correspond to the SD. (**C**) Release of BLT-esterase by CD1-restricted CTLs induced by treatment with Sr^{2+} (*26*). The data shown are representative of three independent experiments, each performed in triplicate. Data are given as the absorbance at 405 nm ± SD. (**D**) Expression of FasL and perforin mRNA by DN and $CD8^+$ CTLs. CTLs (2×10^5) were stimulated with antigen for 12 hours, and total RNA was isolated as described (*27*). cDNA was synthesized and standardized to yield similar amounts of CD3δ PCR product within the linear range of amplification. cDNA specific for FasL (*28*) and perforin (*29*) was amplified by PCR and visualized by autoradiography (*28*). Lane 1: DN.OR; lane 2: DN1; lane 3: DN.LDN4; lane 4: CD8.1; lane 5: CD8.2; and lane 6: CD8.FP1.

completely dependent on cytotoxic granules (*13*).

The question remains as to why the immune response has two virtually independent modes of cytotoxic responses. Lysis of infected macrophages will release intracellular bacteria, thus reducing the reservoir of infected cells. The bacteria will be dispersed and taken up at low multiplicities of infection (MOIs) by activated infiltrating macrophages, which can kill them (*14*). In addition, the process of lysing the infected target cell may directly or indirectly result in the death of the bacteria. To determine whether CD1-restricted T cell activation results in killing of intracellular mycobacteria, we cocultured CTL lines with *M. tuberculosis*–infected $CD1^+$ cells and measured bacterial viability after 18 hours. Whereas four DN, CD1-restricted T cell lines had no effect on the number of colony-forming units (CFUs) of virulent *M. tuberculosis*, both $CD8^+$ CD1-restricted T cell lines examined reduced the number of CFUs by 35 to 50% (Fig. 4). In addition, two human influenza peptide–specific $CD8^+$ CTL lines that cause lysis solely by a granule-dependent mechanism reduced the number of viable mycobacteria by lysing infected macrophages that had been simultaneously pulsed with influenza peptide. Although the percentage reduction of CFUs was within an order of magnitude, *M. tuberculosis* infection in vivo is slow and protracted, and the time of in vitro assay was only 18 hours, so that a cumulative antimicrobial effect mediated by these T cells over time could have a profound effect on the number of bacilli during the course of infection.

These data and a recent study of a murine model (*15*) suggest that the two defined mechanisms of cytotoxicity are associated with distinct phenotypic T cell subsets, yet have differential effects on microbial immunity. Consistent with the findings that Fas-FasL interactions appear to be most relevant to lysis of cells of the immune system itself, this mechanism may function primarily in immune regulation in vivo, particularly in eliminating antigen-expressing APCs, thereby down-regulating immune-mediated tissue injury (*16*). In contrast, the ability of $CD8^+$ CTLs both to lyse infected cells by the granule-dependent mechanism and to kill intracellular *M. tuberculosis* suggests that they may have a special role in resistance to infectious pathogens. The finding in gene-disrupted mouse models that perforin is itself not essential for resolution of mycobacterial infection in vivo (*17*) raises the possibility that the antimicrobial activity may be independent of the lytic process or that there may be additional mediators in the cytotoxic granules, such as granzymes, defensins, or granulysin (*18*). Delineation of the mechanism whereby CD1-restricted CTLs kill intracellular mycobacteria may provide useful insights into mechanisms whereby other types of CTLs contribute to protection against microbial pathogens.

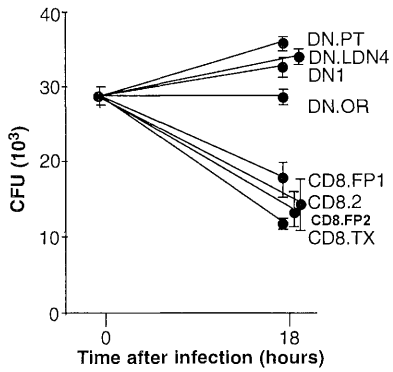

Fig. 4. Perforin, but not Fas-FasL–mediated lysis, inhibits the growth of *M. tuberculosis*. Macrophages infected with live *M. tuberculosis* were coincubated with DN or $CD8^+$ CTLs at an E:T ratio of 10:1. After 18 hours, cells were lysed with saponin to release intracellular bacteria. For the determination of mycobacterial viability, fivefold dilutions of the lysate were plated in duplicate on 7H11 agar plates. CFUs were counted after a 3-week incubation. The results shown are representative of two independent experiments. Error bars correspond to the SEM.

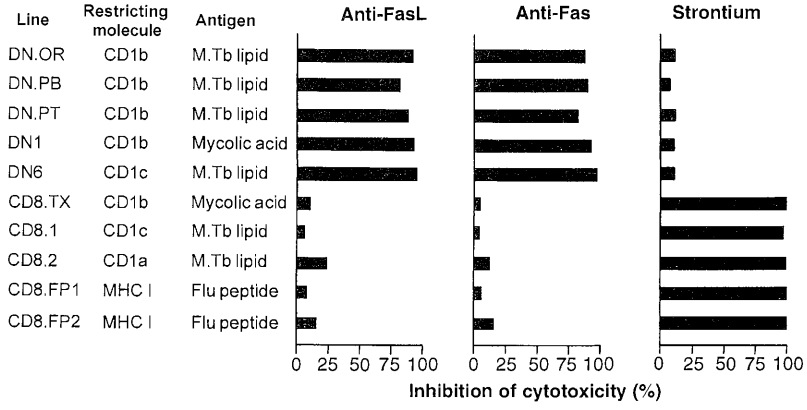

Fig. 3. Subset-dependent mechanisms of cytotoxicity. The ability of blocking antibodies to FasL or Fas and of Sr^{2+} to inhibit antigen-specific cytotoxicity of CD1- or MHC class I–restricted CTLs at an E:T ratio of 10:1 was determined. Inhibition of cytotoxicity was calculated as [(specific cytotoxicity in the absence of inhibitor − specific cytotoxicity in the presence of inhibitor)]/(specific cytotoxicity in the absence of inhibitor).

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11. Tumor necrosis factor–α (TNF-α) induces lysis of a murine fibrosarcoma cell line independently of perforin or Fas-FasL (*19*). This cytokine did not contribute to lysis of the CTLs used in this study because addition of blocking antibody to TNF-α did not inhibit the cytotoxicity.
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25. $CD1^+$ macrophages were pulsed with soluble *M. tuberculosis* extract (5 μg/ml) overnight, detached with 1 mM EDTA, and labeled with 100 μCi of ^{51}Cr for 1 hour. For inhibition of the interaction between FasL and Fas, the assay was done in the presence of blocking antibodies to FasL (*21*) (Pharmingen, San Diego, CA) or Fas (*22*) (Immunotech, Westbrook). Degranulation of the cytotoxic granules was induced by initial treatment of T cells with 25 mM Sr^{2+} (Aldrich, Milwaukee, WI) for 18 hours. ^{51}Cr release was determined after a 4-hour incubation. Expression of Fas on the target cells was not affected by infection with *M. tuberculosis*, as determined by flow cytometry.
26. CTLs (5×10^5) were incubated in the presence of 25 mM Sr^{2+} for 10 hours in a final volume of 1.5 ml. The amount of Nα-CBZ-$_L$-lysine thiobenzyl (BLT)–esterase in the supernatant was determined by the BLT-esterase assay (*23*). The supernatants (20 μl) were coincubated with 35 μl of 1 mM BLT (Sigma), 35 μl of 1 mM 5,5′-dithio-*bis*-(2-nitrobenzoic acid) (Sigma), and 10 μl of a 0.1% Triton X-100 (Sigma) solution. After a 30-min incubation at 37°C, the absorbance at 405 nm was determined.
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Multiple and Ancient Origins of the Domestic Dog

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Mitochondrial DNA control region sequences were analyzed from 162 wolves at 27 localities worldwide and from 140 domestic dogs representing 67 breeds. Sequences from both dogs and wolves showed considerable diversity and supported the hypothesis that wolves were the ancestors of dogs. Most dog sequences belonged to a divergent monophyletic clade sharing no sequences with wolves. The sequence divergence within this clade suggested that dogs originated more than 100,000 years before the present. Associations of dog haplotypes with other wolf lineages indicated episodes of admixture between wolves and dogs. Repeated genetic exchange between dog and wolf populations may have been an important source of variation for artificial selection.

The archaeological record cannot resolve whether domestic dogs originated from a single wolf population or arose from multiple populations at different times (*1*, *2*). However, circumstantial evidence suggests that dogs may have diverse origins (*3*). During most of the late Pleistocene, humans and wolves coexisted over a wide geographic area (*1*), providing ample opportunity for independent domestication events and continued genetic exchange between wolves and dogs. The extreme phenotypic diversity of dogs, even during the early stages of domestication (*1*, *3*, *4*), also suggests a varied genetic heritage. Consequently, the genetic diversity of dogs may have been enriched by multiple founding events, possibly followed by occasional interbreeding with wild wolf populations.

We sequenced portions of the mitochondrial DNA of wolves and domestic dogs. Initially, 261 base pairs (bp) of the left domain of the mitochondrial control region (*5*) were sequenced from 140 dogs representing 67 breeds and five crossbreeds and 162 wolves representing 27 populations from throughout Europe, Asia, and North America (Fig. 1) (*6*). Because all wild species of the genus *Canis* can interbreed (*7*) and thus are potential ancestors of the domestic dog, five coyotes (*Canis latrans*) and two golden, two black-backed, and eight Simien jackals (*C. aureus*, *C. mesomelas*, and *C. simensis*, respectively) were also sequenced.

The control region of wolves and dogs was highly polymorphic (Fig. 1). We found 27 wolf haplotypes that differed on average by 5.31 ± 0.11 (±SE) substitutions (2.10 ± 0.04%), with a maximum of 10 substitutions (3.95%). The distribution of wolf haplotypes demonstrated geographic specificity, with most localities containing haplotypes unique to a particular region (Fig. 1). Four haplotypes (W2, W7, W14, and W22) had a widespread distribution. In dogs, 26 haplotypes were found. Only haplotype D6 also occurred in

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