hold silicone glue and allowed to dry for at least 24 hours. The resulting "membrane dish" had a surface of 4.15 cm^2. Membrane dishes of the same size were also prepared by casting 0.5 ml of a mixture of ten parts Sylgard 184 silicone elastomer and one part Sylgard 184 curing agent (Dow Corning) into 35-mm plastic culture dishes and allowing them to polymerize at 65°C overnight. When the membranes were still sticky, but firm, the silicone ring was allowed to polymerize into the membrane to avoid the use of glue. Sylgard-type membranes were 0.23 mm thick and optically clear. Both types of membranes were sterilized and coated overnight with bovine plasma fibronectin (200 μg/ml).

4. Type II cells were isolated from pathogen-free adult male Sprague-Dawley rats (Bantin-Kingman) [L. G. Dobbs, R. F. Gonzalez, M. C. Williams, *Am. Rev. Respir. Dis.* **134**, 141 (1986)]. We cultured 2.5×10^5 cells/ml on each membrane in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (University of California at San Francisco Cell Culture Facility), 2 mM glutamine, penicillin (100 U/ml), and gentamicin (50 μg/ml). [^{3}H]Choline was obtained from Amersham Corporation. Secretion of [^{3}H]PC was measured as previously described [L. G. Dobbs, R. F. Gonzalez, L. A. Marinari, E. J. Mescher, S. Hawgood, *Biochim. Biophys. Acta* **877**, 305 (1986)]. Medium and cells were harvested and centrifuged, and lipids were extracted [J. Folch, M. Lees, G. H. S. Stanley, *J. Biol. Chem.* **226**, 497 (1957)]. Radioactivity was measured in a liquid scintillation counter (Beckman Instruments LS 7500).
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6. The stretching devices we constructed provide space for two groups of three membranes per device. Each group of three membranes can be stretched independently. Thus three experimental membranes (stretched) were compared to three control membranes (unstretched) under equivalent conditions. Membranes were held in place by an acrylic top plate (2 cm high) with round borings forming the wells. Pressure was applied manually with a syringe and transduced to the membranes by fluid; the amount of stretch was controlled by the volume of fluid added.
7. Height was measured from the middle of the distended elastic membrane. Volume added was plotted against the calculated increase in membrane surface area. The volume:MSA relationship was reproducible and linear over the range used. It was nearly identical for both types of membranes.
8. Cellular surface area is defined here as the area that is occupied by the cell in two dimensions as viewed through a microscope and not as a true three-dimensional measurement of cellular surface. Cellular surface area was measured by digitizing cell area on photographs (Calcomp 6000). Although there was cell-to-cell variation in CSA/MSA ratio, this ratio was higher (0.75 ± 0.36; mean ± SD, $n = 20$) for silastic sheeting than it was for the Sylgard membranes (0.40 ± 0.11, $n = 6$), possibly because of the smoother Sylgard surface. We do not know the reason for the difference between MSA and CSA. Cell attachment sites to fibronectin may be broken when cells are stretched. Alternatively, the cells or the matrix may slide on the membrane, resulting in less distension of the cells compared to the membrane.
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10. Data from 23 of 300 stretched and 13 of 210 unstretched membranes were discarded because LDH release was greater than 1%; LDH released from cells on these discarded membranes averaged 2.2 ± 1.6% for stretched and 3.2 ± 3.4% for unstretched membranes.
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21. Cyclic AMP content was determined with a radioimmunoassay (Rainen, Du Pont) after extraction of the cells at 4°C with 0.1 M HCl and rapid freezing. Results were similar to previously published observations [E. J. Mescher, L. G. Dobbs, R. J. Mason, *Exp. Lung Res.* **5**, 173 (1983)]. [Ca^{2+}]$_i$ was measured with an ACAS 470 laser cytometer (Meridian Instruments Inc.). Cells cultured on Sylgard membranes were incubated for 1 hour with medium containing 2 μM Indo-1-AM (Molecular Probes Inc.) and transferred to a stretching chamber (37°C) adapted to the ACAS stage. To measure rapid changes in [Ca^{2+}]$_i$, we used linescans (120 points; step size 0.25 μm) to measure [Ca^{2+}]$_i$ in a single cell over time (additional image scans correlated well with the [Ca^{2+}]$_i$ values obtained from linescans). The ratio of fluorescence at 485 nm and 405 nm was calculated for each point. A calibration curve relating [Ca^{2+}]$_i$ to fluorescence was constructed according to the method described [M. H. Wade and S. A. McQuiston, *ACAS 470 Training Manual* (Meridian Instruments Inc., Okemos, MI, 1988), p. 37]. [Ca^{2+}]$_i$ was measured for various lengths of time before stretching. Measurement of [Ca^{2+}]$_i$ during stretch was not feasible because the focal plane changed when the membrane was distended. Stretch was carried out by adding warmed fluid to the chamber with a syringe and followed by immediate relaxation. The same cell was quickly identified and centered, and measurement of fluorescence was restarted as quickly as possible. Stretch and relaxation were usually completed in 12 s. The time required to recenter the cell and restart the instrument was more variable; time from start of stretch to restart of measurement was 21.7 ± 8.9 s for experiments in Ca^{2+}-Mg^{2+}–containing medium and 19.4 ± 4.9 s for experiments in Ca^{2+}-Mg^{2+}–free medium. The background determined from a cell-free area of the membrane was subtracted and [Ca^{2+}]$_i$ from linescans was averaged.
22. Supported in part by grants from the NIH (HL-24075 and HL-34356) and Deutsche Forschungsgemeinschaft. We thank J. Nellenbogen and D. Johnson for expert technical assistance, J. R. Wright for help in preparing rat SP-A, and J. A. Clements, M. C. Williams, J. Goerke, and M. J. Fulwyler for helpful discussions and suggestions. We also thank Burroughs-Wellcome Inc. for their gift of BW 755C.

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Recognition by Human $V_\gamma 9/V_\delta 2$ T Cells of a GroEL Homolog on Daudi Burkitt's Lymphoma Cells

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All human γδ T cells coexpressing the products of the variable (V) region T cell receptor (TCR) gene segments $V_\gamma 9$ and $V_\delta 2$ recognize antigens from some mycobacterial extracts and Daudi cells. Exogenous and endogenous ligands on the cell surface, homologous to the groEL heat shock family, induced reactivities that resembled superantigen responses in this major subset of human peripheral blood γδ T cells. Stimulation of human $V_\gamma 9/V_\delta 2$ T cells is not restricted by human leukocyte antigens (HLA), including nonpolymorphic β_2-microglobulin (β_2M)–associated class Ib molecules. These data may be important for understanding the role of γδ T cells in autoimmunity and in responses to microorganisms and tumors.

A SUBSET OF T CELLS EXPRESS THE γδ T cell receptor (TCR) (*1*) and have limited variable (V) region repertoire (*2*). Antigens that stimulate γδ T cells are only now being identified (*3*), and it is not clear whether they need to be processed and presented as is the case for αβ T cells (*4*). The nonclassical major histocompatibility complex (MHC) class Ib molecules also have limited polymorphism and have been proposed as antigen-presenting molecules for γδ T cells (*5*). Both murine (*6, 7*) and human (*8*) γδ T cells can respond to mycobacterial extracts. In the mouse, the 65-kD mycobacterial heat shock protein (hsp65) and peptides from it can stimulate γδ T cells (*6*). Hsp65 is homologous to the groEL protein of *Escherichia coli* and similar stress proteins in prokaryotic and eukaryotic cells (*9, 10*). Examination of over 2000 human γδ T cell clones revealed that all of those expressing both $V_\gamma 9$ and $V_\delta 2$ (*11, 12*) are particularly efficient killers of the Daudi Burkitt's lymphoma cell line (*13*). In this study, we examined the proliferative responses of human γδ T cells to endogenous and exogenous antigens. The proliferative response of human $V_\gamma 9/V_\delta 2$ T cells to Daudi cells and bacterial extracts could be inhibited by antiserum to the mammalian groEL related heat shock proteins (hsp58 or hsp60) (*14*). This serum immunoprecipitated a molecule of corresponding molecular size from the surface of Daudi cells. We

found that, unlike previous results, β_2M binding MHC proteins are unlikely to be involved in the presentation of antigen to human $V_\gamma 9/V_\delta 2$ T cells.

Initially, we tested our clones for their ability to proliferate in response to various cell lines and to mycobacteria (Table 1). The $V_\gamma 9/V_\delta 2$ clones, but none of the others, were stimulated by the Daudi cell line and by some preparations of sonicated mycobacteria (*15*), when presented by appropriate antigen-presenting cells (APCs). This was the first exposure of the clones to Daudi cells or mycobacterial antigens in vitro (*16*). Lymphoblastoid B cell lines (LCLs) other than Daudi cells stimulated the $V_\gamma 9/V_\delta 2$ clones only in the presence of the mycobacterial extracts (Table 1). However, intracellular processing of the mycobacterial extracts was not necessary because paraformaldehyde fixation of the APCs did not eliminate stimulation by the mycobacterial antigens (Fig. 1). Allogenic mutant LCLs that do not express polymorphic human leukocyte antigens (HLA) class I (LCL 721.221) or class II (LCL 721.180) determinants (*17*), the parent nonmutated LCL 721, and Daudi cells (*18*) presented the mycobacterial antigens to the $V_\gamma 9/V_\delta 2$ clones (Fig. 1). Daudi cells do not express β_2M-associated HLA class I proteins because the translated class I α chains in Daudi are not transported to the cell surface due to a mutation of the β_2M mRNA initiation codon (*18*). A Daudi variant that expresses cell surface HLA class I (*18*) was as stimulatory for the human $V_\gamma 9/V_\delta 2$ T cells as the parent Daudi cell line (Table 1). Thus, the response to Daudi was not associated with the absence of HLA class I antigens on the cell surface. However, other APCs (such as K562, Molt4, or Raji cells), independent of their surface class I or class II expression, did not present mycobacterial antigens to the $\gamma\delta$ T cells (Fig. 1). Daudi cells presented the mycobacterial antigens, even after adaptation to serum-free medium and fixation (Fig. 1). This excludes the possibility that serum β_2M allowed the transport of HLA class I molecules to the Daudi cell surface. Thus, the antigens for the $V_\gamma 9/V_\delta 2$ T cells do not require presentation by conventional polymorphic or β_2M-associated MHC molecules. This would be consistent with the absence of CD4 or CD8 accessory molecules on most $\gamma\delta$ T cells, and the normal development of $\gamma\delta$ T cells in β_2M-deficient mice (*19*).

We then examined the reactivity of fresh peripheral blood lymphocytes (PBL) to the antigens that stimulate the $V_\gamma 9/V_\delta 2$ clones. As determined by flow cytometry, both the mycobacterial preparation and Daudi cells, but not Candida antigens or Raji cells, induced strong proliferation of human peripheral blood $V_\gamma 9/V_\delta 2$ T cells (Fig. 2). This indicates that the immunological reactivity of $V_\gamma 9/V_\delta 2$ T cells was not a phenomenon detected only after in vitro culture of $\gamma\delta$ T cell clones with interleukin-2 (IL-2). Human $\gamma\delta$ T cells also expanded and $V_\gamma 9/V_\delta 2$ clones were stimulated by some *E. coli* extracts (*20*). The reactivity of $V_\gamma 9/V_\delta 2$ T cells to Daudi and mycobacterial antigens was detected in both tuberculin skin test–negative and –positive individuals. Daudi stimulation of umbilical cord blood mononuclear cells [less than 0.1% $\gamma\delta$ T cells, predominantly $V\delta1^+$ (*12, 21*)], induced expansion of $\gamma\delta$ T cells that coexpressed $V_\gamma 9/V_\delta 2$ genes (50 to 80 percent $V_\gamma 9/V_\delta 2$ T cells 3 weeks after two sequential exposures to Daudi cells in vitro). In addition, $\gamma\delta$ T cells from rhesus monkeys [reactive with the monoclonal antibody (MAb) TCRδ1 to the human $\gamma\delta$ TCR] proliferate in response to

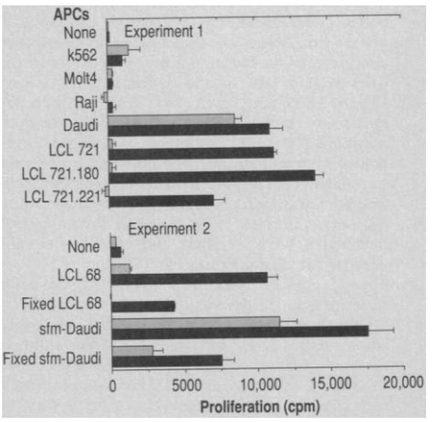

Fig. 1. Presentation of mycobacterial antigen to $V_\gamma 9/V_\delta 2$ T cells by different APCs. Proliferation ([^{3}H]thymidine incorporation as mean counts per minute ± standard error of the mean of quadruplicates) of the clone GPC4 to diverse APCs in the absence (shaded bars) or presence (filled bars) of sonicated H37Rv mycobacteria (*15*). The proliferative assays were performed as described (*30*). APCs were K562, Molt4, Raji, Daudi cells, LCL 68 (the autologous LCL for clone GPC4), LCL 721, and variants from LCL 721 that have partial deletions of the chromosome 6, LCLs 721.180 and 721.221. LCL 721.180 has reduced levels of HLA class I on the surface and does not express class II encoded by the *DR*, *DP*, and *DQ* loci, whereas LCL 721.221 does not express HLA class I antigens A, B, and C (*17*). Daudi cells that had been adapted to serum-free medium (HL-1, Ventrex Laboratories) before the assay are designated sfm-Daudi. All proliferative assays were performed in the presence of 10% human serum. LCL 68 and sfm-Daudi cells were fixed by 5-min incubations in 0.15% paraformaldehyde (*23*). Similar results with these APCs were obtained with other $V_\gamma 9/V_\delta 2$ clones in six independent experiments. The antigen presenting capacity of LCL 721, LCL 721.180, and LCL 721.221 were comparable, whereas some other LCL lines were less effective as APCs. The sfm-Daudi cells were lysed by the $V_\gamma 9/V_\delta 2$ clones equally well as the Daudi cells, grown in serum containing medium, even when the cytotoxicity assays were performed in serum-free medium.

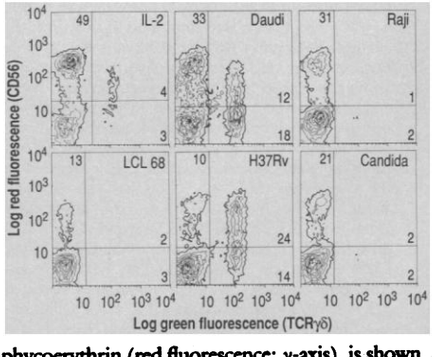

Fig. 2. Flow cytometric analysis of the proliferation of human peripheral blood $V_\gamma 9/V_\delta 2$ T cells after stimulation with Daudi cells or mycobacterial antigens. PBLs (10^6/ml) were stimulated with IL-2 (100 units/ml), irradiated Daudi, Raji, or LCL 68 cells (all at 2×10^5/ml), extracts from H37Rv mycobacteria (*15*) or Candida. On day 7, cells from all cultures were labeled with MAbs and 10,000 events per sample were analyzed on a FACStar^{Plus} (Becton-Dickinson) using four decade log signal amplification. All viable cells were gated on forward scatter and by exclusion of propidium iodide (1 μg/ml). Two color analysis with MAbs to the $\gamma\delta$ TCR (TCRδ1), conjugated to fluorescein isothiocyanate (green fluorescence; *x*-axis), and MAbs to CD56 (Leu19), conjugated to phycoerythrin (red fluorescence; *y*-axis), is shown. A marked expansion of human peripheral blood $\gamma\delta$ T cells (right upper and right lower quadrants) was seen after stimulation with Daudi and the H37Rv preparation but by none of the other antigens. The cells from the left upper quadrants were identified as NK cells and in the left lower quadrants as $\alpha\beta$ T cells, by parallel staining with other MAbs. The percentages of cells in the quadrants are indicated. The $\gamma\delta$ T cells expanded by Daudi and mycobacteria coexpressed Vγ9 and Vδ2, but they did not express Vδ1, as determined by the MAbs described (*11*).

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Daudi (but not Raji) cells (*22*). These observations suggest that the reactivity of human γδ T cells to Daudi is displayed early during ontogeny and is conserved during phylogeny.

Because hsps are a major antigenic component of mycobacterial extracts, we tested polyclonal rabbit antisera specific for the hsp70 and groEL hsp families for inhibition of the proliferative response of the $V_\gamma9/V_\delta2$ T cells to Daudi. The proliferation of the peripheral blood γδ T cells and the $V_\gamma9/V_\delta2$ clones to Daudi was suppressed by the hsp58-specific antiserum (*14*), but not by hsp70 antisera (*14, 23*) or nonimmune sera (Fig. 3). Thus, $V_\gamma9/V_\delta2$ T cells recognize a ligand on the cell surface of Daudi cells related to the groEL hsp family. This is consistent with the specific recognition of Daudi cells by $V_\gamma9/V_\delta2$ clones in cytotoxicity assays (*13*) and with the lack of proliferation-inducing activity in cell-free Daudi culture supernatants. Also, the expansion of human γδ cells from PBL induced by bacterial antigens could be markedly inhibited by the hsp58 antiserum (Fig. 3), suggesting that the $V_\gamma9/V_\delta2$ T cells recognize related endogenous ligands on Daudi and exogenous ligands from some bacterial preparations (*14*).

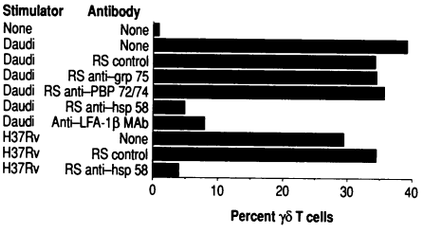

Fig. 3. The proliferation of peripheral blood γδ T cells, induced by Daudi and mycobacteria, can be inhibited by antiserum against the mitochondrial hsp58. PBL were activated with irradiated Daudi cells and sonicated mycobacteria (H37Rv) in the absence or presence of hsp58 antiserum and control antibodies. The antibodies were control nonimmune rabbit sera, rabbit antisera to the hsp70 family [PBP 72/74 (*23*) and grp75 (*14*)], rabbit antiserum specific to hsp58 (*14*), and mouse MAb to LFA-1β (MAb TS1/18) (*31*). The cultures were analyzed by flow cytometry. Only the hsp58 antiserum and the MAb to LFA-1β inhibited the expansion of γδ T cells. The hsp58 antiserum could also inhibit the proliferation of peripheral blood γδ T cells induced by *E. coli* extracts (*14*), but did not block the stimulation of γδ T cells by IL-2 or MAbs to CD3, the expansion of NK cells by Daudi, or the proliferation of Daudi cells. All rabbit sera were tested at a 1:40 dilution. The data were confirmed in three independent experiments.

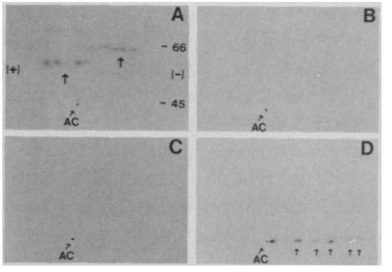

Fig. 4. Hsp58 can be detected on the cell surface of Daudi whereas HLA class I α chains are only detectable in the cytoplasm. (**A**) Surface-labeled Daudi cells, immunoprecipitated with the hsp58 antiserum (*14*); (**B**) surface-labeled Daudi cells, immunoprecipitated with control, nonimmune rabbit serum; (**C**) surface-labeled Daudi cells, immunoprecipitated with MAb Q1/28 that binds to the HLA class I α chains in the absence of β_2M (*32*); (**D**) metabolically labeled Daudi cells, immunoprecipitated with MAb Q1/28. AC is the position of actin; purified actin was added to the samples to aid in orientation of the spots in the second dimension. The gels are oriented from acidic (+) to basic (−). The arrows point to the immunoprecipitated proteins at 58 and 66 kD in (A) and to the HLA class I isoforms at 44 kD in (D). For surface labeling, 2.5×10^7 Daudi cells were surface-iodinated with ^{125}I (1.0 mCi) with Iodogen (Pierce Chemicals) (*33*). The metabolic labeling with [^{35}S]methionine, immunoprecipitations, and 2-D gel analysis were done as described (*34*).

We used the hsp58-specific antiserum to immunoprecipitate groEL-related molecules from surface-iodinated Daudi cell lysates. Two-dimensional electrophoresis of the immunoprecipitate revealed 58-kD and 66-kD molecules (Fig. 4). A similar complex of hsp58 and an hsp70 molecule is immunoprecipitated from the mitochondria of internally labeled HeLa cells, using the same hsp58 antiserum (*24*). To control for the surface specificity of the iodination, we immunoprecipitated with a MAb to class I α chain. We did not detect any iodinated class I α chains (Fig. 4C) in the same Daudi lysate, whereas the HLA class I α chains were precipitated from the cytoplasm of biosynthetically labeled Daudi cells (Fig. 4D) and from iodinated cells expressing HLA class I on their surface. Moreover, no ^{125}I-labeled actin was detected in the cell lysate from surface-labeled Daudi cells (Fig. 4, A to C) (actin binds nonspecifically to the immunoglobulin coated beads, used for the immunoprecipitation), confirming the specificity of the surface iodination procedure.

Although groEL hsps are intracellular proteins and as yet have not been characterized on the cell surface (*9, 10*), the reactivity of T cells may provide a very sensitive method of their surface detection (*25, 26*). The groEL-related protein we detected at

Table 1. Specific proliferation of $V_\gamma9/V_\delta2$ clones to Daudi cells and mycobacterial extract. Numbers represent [^3H]thymidine incorporation (mean counts per minute ± standard error of the mean of quadruplicates). The proliferative assays are described (*30*). The expression of the V regions was determined with MAbs to $V_\gamma9$, $V_\delta2$, and $V_\delta1$ (*11*). Representative are $V_\gamma9^+/V_\delta1^-/V_\delta2^+$ T cell clones BT60, FW1, GPE5, and GPC4, other γδ T cell clones TS4B ($V_\gamma9^-/V_\delta1^+/V_\delta2^-$)‡ and PJ4 ($V_\gamma9^-/V_\delta1^-/V_\delta2^-$), and αβ TCR clone AD4. Proliferation was measured in medium alone, in the presence of IL-2 (500 units/ml), surface immobilized MAb to CD3 MAb (OKT3), or diverse target cell lines(Daudi, β_2M-Daudi, Raji, LCL 68). β_2M-Daudi is the HLA class I^+ Daudi variant after transfection with the mouse β_2M gene (*18*); LCL 68 is representative for other LCL lines studied. Stimulation by LCL 68 was measured in the absence or presence of the H37Rv mycobacterial antigens (*15*) or Candida antigens at concentrations determined as optimal in preliminary experiments. Control PBL cultures primed to H37Rv and Candida showed specificity for these antigens. NT = not tested. The $V_\gamma9/V_\delta2$ clones but not the other clones lysed Daudi cells in cytotoxicity assays although all clones could lyse susceptible targets (*13*).

Simulator	Responder clone						
	BT60	FW1	GPE5	GPC4	PJ4	TS4B	AD4
Medium	537 ± 71	328 ± 91	468 ± 171	544 ± 77	285 ± 14	779 ± 26	644 ± 17
IL-2	NT	7,476 ± 136	13,350 ± 161	11,897 ± 156	62,802 ± 881	8,276 ± 257	18,795 ± 271
Anti-CD3 MAb	19,874 ± 1,973	13,103 ± 1,791	12,602 ± 508	10,951 ± 252	14,484 ± 580	17,593 ± 417	9,252 ± 103
Daudi	16,330 ± 551	6,765 ± 198	12,784 ± 231	8,968 ± 138	1,197 ± 37	792 ± 206	942 ± 103
β_2M-Daudi	NT	7,017 ± 200	14,253 ± 783	9,397 ± 242	1,500 ± 156	NT	328 ± 32
Raji	585 ± 275	300 ± 115	812 ± 353	423 ± 35	1,281 ± 105	772 ± 186	233 ± 92
LCL 68	903 ± 119	280 ± 127	521 ± 196	323 ± 26	5,291 ± 316	872 ± 76	1,421 ± 23
LCL 68 + C	NT	261 ± 238	123 ± 37	78 ± 31	NT	923 ± 75	1,041 ± 49
LCL 68 + H	8,930 ± 1,866	6,160 ± 149	16,752 ± 566	12,427 ± 513	3,122 ± 177	624 ± 85	1,166 ± 63

*C, Candida; H, H37Rv

the cell surface could be a novel member of the groEL family or the known form of groEL could be transported to the cell surface with another molecule. An MHC class I–like molecule not binding β_2M (*27*) may function as a transport mechanism for groEL. It is also possible that the 66-kD molecule associated with hsp58 on the cell surface (Fig. 3A) might be involved in the transport of groEL to the cell membrane. Molecules that bind and present endogenous antigens might also present homologous exogenous antigens to γδ T cells. Such determinants could be expressed at low levels (*26*) on APCs that stimulated γδ T cells in the presence, but not in the absence, of exogenous antigens.

Thus, all human T cells coexpressing $V_\gamma 9$ and $V_\delta 2$ genes proliferate in response to Daudi cells and to certain mycobacterial extracts, whereas the Daudi nonreactive γδ T cells [that is, the $V_\delta 1$ subset (*12*)] do not recognize mycobacteria. The ligands recognized by the $V_\gamma 9/V_\delta 2$ T cells appear to be homologs of the groEL hsp family, either as endogenous ligands, expressed on the cell surface of Daudi, or as exogenous bacterial antigens that can be presented by APCs without antigen processing. Recognition by human $V_\gamma 9/V_\delta 2$ T cells of these antigens does not require antigen presentation by β_2M-associated HLA class I or polymorphic HLA class II molecules. Only a few preparations of crude mycobacterial antigens stimulate human γδ T cells effectively (*15*). Purified hsp65 antigens (*15*), including the groEL peptides that stimulated murine γδ T cells hybridomas (*6*), did not stimulate our human γδ T cells. Our results suggest that the stimulatory epitopes for human $V_\gamma 9/V_\delta 2$ T cells may depend on the conformation of groEL molecules or their peptide fragments and on suitable presentation on the cell surface. Ineffective antigen presentation to γδ T cells (*6*) could explain why only few investigators found purified hsp65 preparations stimulatory for human mycobacteria-reactive γδ T cells (*8*).

The proliferative responses of all $V_\gamma 9/V_\delta 2$ T cells, but not other γδ T cells, to Daudi cells and mycobacterial antigens support the idea of TCR-mediated antigen recognition. Because human peripheral blood γδ T cells have extensive junctional diversity of both the γ and δ chains (*2*), it is unlikely that this recognition is determined by the junctional diversity. Coexpression of $V_\gamma 9$ and $V_\delta 2$ genes seems to be required for antigen responsiveness to Daudi and mycobacteria (*28*). The requirement for specific V regions, irrespective of the diversity of junctional segments and the proliferation of the whole T cell subset in response to the same exogenous and endogenous antigens resemble superantigen responses of murine $V_\beta 8$ T cells to staphylococcal enterotoxins and Mls (*29*). Peripheral human $V_\gamma 9/V_\delta 2$ cells are selectively expanded in the first 10 years after birth, but thymic $V_\gamma 9/V_\delta 2$ T cells remain only a minor fraction of γδ T cells (*12, 21*). Our results indicate that endogenous and exogenous ligands, homologous to groEL hsps, could be responsible for this in vivo expansion (*21*). Recognition of groEL-related proteins by T cells may play a role in the pathogenesis of infectious and autoimmune diseases (*9, 10, 25*) and could explain the reactivity to the Daudi lymphoma. Therefore, the $V_\gamma 9/V_\delta 2$ T cell subset may participate in diseases of infectious, autoimmune, and neoplastic origins.

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16. The clones were derived by sorting human γδ T cells from peripheral blood with various MAbs (*11*) to the γδ TCR with a FACStar^{Plus} (Becton-Dickinson). The sorted cells were cloned and expanded with LCL (such as LCL 68) and PBL feeder cells as described (*13*), but never with Daudi cells or bacterial antigens.
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28. All $V_\gamma 9/V_\delta 2$ T cells react strongly to these antigens, whereas the majority of $V_\delta 1^+$ (including $V_\gamma 9/V_\delta 1$) T cells do not. Preliminary results suggest that very rare $V_\gamma 9^+/V_\delta 1^+$ T cell clones [as determined by MAbs (*11*)] may react with lower affinity.
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Diet-Induced Hypercholesterolemia in Mice: Prevention by Overexpression of LDL Receptors

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The current studies were designed to determine whether chronic overexpression of low density lipoprotein (LDL) receptors in the liver would protect mice from the increase in plasma LDL-cholesterol that is induced by high-fat diets. A line of transgenic mice was studied that express the human LDL receptor gene in the liver under control of the transferrin promoter. When fed a diet containing cholesterol, saturated fat, and bile acids for 3 weeks, the transgenic mice, in contrast to normal mice, did not develop a detectable increase in plasma LDL. The current data indicate that unregulated overexpression of LDL receptors can protect against diet-induced hypercholesterolemia in mice.

DIETS HIGH IN CHOLESTEROL AND saturated fats increase the plasma level of low density lipoprotein (LDL), owing to a combination of increased cholesterol content of precursor particles, increased synthesis of particles, and decreased hepatic degradation (*1*). Degradation is mediated by LDL receptors, most of which are located on hepatocytes (*2*). When high-cholesterol diets are ingested, hepatic LDL receptors are suppressed, and this contributes to the subsequent elevation in plasma LDL levels (*3–5*).

The question arises as to whether forced overexpression of LDL receptors in liver would ameliorate or prevent diet-induced hypercholesterolemia. Although a stimulation of LDL receptor production is one means by which drugs lower plasma LDL concentrations (*2*), it is not known whether such an approach would succeed during feeding of a high-fat diet. Now we attempt to answer this question by using transgenic mice that abnormally overexpress human LDL receptors.

We have previously achieved overexpression of LDL receptors by injecting mouse eggs with a cDNA encoding the human LDL receptor under control of the mouse metallothionein-I promoter (designated transgene-1) (*6*). Mice expressing this transgene had high levels of LDL receptor activity in liver when the metallothionein promoter was induced through the administration of $CdSO_4$. As a consequence, the hepatic uptake of LDL increased. When the animals ate a low-fat diet, the plasma concentration of the apoproteins (apo) B and E fell to virtually undetectable levels (*6*). We could not use transgene-1 for long-term studies, since high-level expression of this transgene requires induction with $CdSO_4$ (*6*), and chronic treatment with such heavy metals is toxic to the animals. To circumvent this problem, we have established a new strain of transgenic mice that express a human LDL receptor minigene at high levels without the need for metal induction. The human LDL receptor minigene, designated transgene-3 (Fig. 1), is contained on a 15.5-kb Not I fragment that includes 3 kb of the mouse transferrin promoter and 27 bp of 5′ untranslated region from the human LDL receptor gene followed by the first four exons and introns of this gene. This sequence is followed by contiguous exons 5 to 18 contained in a single fragment derived from the LDL receptor cDNA. As with other transgene constructions (*7*), the minigene gave much higher levels of expression than did the earlier transgene that contained only a cDNA sequence with no intron sequences (*6*). Previous studies in transgenic mice showed that the 3-kb transferrin promoter segment used in the current study gave expression of human growth hormone that was high in liver and low in brain and kidney (*8*). We confirmed high-level expression of the human LDL receptor in the transgenic mouse livers by blot hybridization of mRNA, immunoblot analysis of receptor protein, and immunofluorescence (*9*). Expression of the human receptor protein in the liver of the transgenic mice was four to five times as high as that of the endogenous receptor protein in the normal mouse liver.

When maintained on a normal laboratory diet (*10*), mice expressing LDL receptor

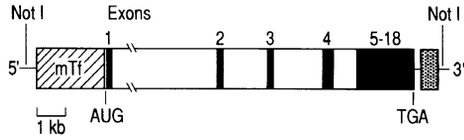

Fig. 1. Human LDL receptor transgene-3. The mouse transferrin (mTf) promoter and transcription initiation site were contained in a 3-kb Bam HI fragment (striped box) derived from the plasmid that includes mTf and human growth hormone (pmTf-hGH) (*8*). This fragment was fused to a 12.5-kb fragment containing a human LDL receptor minigene (*20*) and the transcription termination signal from the human growth hormone gene (stippled box) (*6*). Exons 1 to 18 and introns 1 to 4 are indicated by filled-in and open boxes, respectively. The construct contained 27 bp of 5′ untranslated region from the human LDL receptor gene (*21*). The thin lines represent polylinker sequences. A total of 436 eggs from C57BL/6 × SJL F_2 hybrid mice were microinjected with transgene-3 contained in a 15.5-kb Not I fragment and transferred into pseudopregnant females (*22*). Among 60 offspring, 23 (38%) contained the transgene as determined by dot-blot hybridization of DNA from tail tissues. Of these 23 mice, 6 showed a chronic absence (<1 mg/dl) of apo B-100 in plasma as measured by rocket immunoelectrophoresis (*6*). A strain of heterozygous mice derived from founders (line 212-5) was used in this study. Sibling mice that lacked the transgene (as determined by dot-blot hybridization) were used as controls.

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