Constitutive Expression of a groEL-related Protein
on the Surface of Human \( \gamma/\delta \) Cells

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Summary

Rabbit antibodies to hsp58 (P1), the human homologue of the Escherichia coli stress protein groEL, react specifically in indirect immunofluorescence and complement-dependent microcytotoxicity experiments with a cell surface antigen expressed constitutively by T cell lines bearing \( \gamma/\delta \) receptors. This anti-hsp58-reactive antigen is not demonstrable on T cells that express \( \alpha/\beta \) receptors or on various cells that lack T cell receptors. Certain evidence was obtained to suggest that the target antigen on the surface of \( \gamma/\delta \) T cells is a \( \sim 77 \)-kD protein distinct from intracellular hsp58 and known members of the hsp70 stress protein family. While the exact nature and significance of this anti-hsp58-reactive protein remain to be determined, these data may help to clarify the roles of groEL-related stress proteins and \( \gamma/\delta \) cells that recognize groEL homologues in immunologic defense against infection and in autoimmune disease.

Homologues of the highly conserved Escherichia coli stress protein groEL function as chaperones and may be fundamentally important in the basic immune processes operant in host defense against infection and autoimmunity (reviewed in references 1 and 2). For example, members of this family are immunodominant antigens of bacteria and mycobacteria, exhibit special reactivity with T cells bearing the TCR-\( \gamma/\delta \) CD3 complex (3–7), and have been implicated as inciting antigens in various disorders characterized by T cell autoreactivity, including arthritis, trachoma, and insulin-dependent diabetes. Such observations, together with evidence for bias of the neonatal thymocyte repertoire toward reactivity with a mycobacterial groEL homologue in the mouse (4), have led to speculation that a subset of \( \gamma/\delta \) cells able to recognize groEL-related proteins represents a rapid-response first line of defense against infections, and that crossreactivity with autologous stress proteins in this family, perhaps triggered by common infectious organisms, results in autoaggressive T cell responses and inflammatory tissue injury.

Of special interest is the observation of O'Brien et al. (4) that neonatal thymus \( \gamma/\delta \) hybridomas, which recognize a mycobacterial groEL homologue, hsp65, are self-reactive, i.e., exhibit unusual “spontaneous” TCR-dependent IL-2 secretion. These investigators proposed that this self-reactivity involves the surface expression of mycobacterial hsp65-related autologous stress protein peptides (8). A constitutively expressed mitochondrial stress protein in man has been identified as a highly conserved homologue of groEL and mycobacterial hsp65 (9, 10). In the present investigation, a \( \sim 77 \)-kD molecule antigenically related to hsp58 was shown to be expressed constitutively on cells with TCR-\( \gamma/\delta \), but not on peripheral T cells or various T cell lines that express TCR-\( \alpha/\beta \) or that lack TCRs.

Materials and Methods

Cells. Normal human PBMC were prepared by flotation on lymphocyte separation medium (Litton Bionetics, Kensington, MD). Human cell lines were cultured in RPMI 1640 containing 10% FCS, glutamine, and antibiotics. Cell lines included: E6-1, a Jurkat T cell line expressing TCR-\( \alpha/\beta \); PEER, a leukemia T cell line expressing TCR-\( \gamma/\delta \); JRT3-T3.5, a mutant Jurkat line that does not express TCRs (11); MOLT-4, a leukemia cell line that does not transcribe TCR \( \alpha \) chain genes (12); HSB-2, a primitive T cell line that lacks TCRs and most of the other antigens expressed by peripheral T cells; and the nonlymphoid line HL60, THP-1, and U937. In some experiments, cells were subjected to heat shock at 43°C for 60 min followed by culture for 18 h at 37°C.

Special Immunological Reagents. Preparation and characterization of rabbit anti-hsp58 has been described previously (9). Rabbit antiserum to P1, another mammalian homologue of groEL isolated from Chinese hamster ovary cells (13), was a generous gift of Dr. R. Gupta, McMaster University, Hamilton, Ontario. Antibodies to members of the hsp70 family of stress proteins included mouse monoclonals N4, N6 F3-3, N15, N21, and C92 (constitu-
tive and highly inducible 72–73-kD proteins) and rabbit antiserum specific for the 75-kD glucose-regulated protein (9, 14) (W.J. Welsh, unpublished data). WT31 (anti-TCR-α/β framework) and δ1 (anti-TCR-γ chain) were purchased from Becton Dickinson & Co., Mountain View, CA, and T cell Sciences, Cambridge, MA, respectively. Normal rabbit serum, rabbit anti-BSA and isotype-matched mouse myeloma proteins were used as control reagents. Microcytotoxicity and indirect immunofluorescence/flow cytometry were performed as described in detail previously (15, 16).

Antigen Substrate. E6-1 or PEER cells (4 × 10⁸) suspended in Laemmli sample buffer were disrupted by sonication (W-350 Sonifer; Heat Systems-Ultrasonics, Inc., Farmingdale, NY). For some experiments, 10⁶ cells in 1 mM sodium bicarbonate were homogenized (Brinkmann Instruments Co., Westbury, NY) for preparation of plasma membranes by aqueous two-phase partition according to the technique of Navas et al. (17). Plasma membrane preparations were enriched ≈32-fold for HLA class I antigens relative to whole cell lysates.

Electrophoresis and Immunoblotting. Cell lysates or enriched plasma membranes were analyzed by SDS-PAGE (8% gels, reduced and nonreduced) and immunoblotting with appropriate dilutions of rabbit anti-hsp58 or other antibodies to stress proteins, as described in detail previously (16).

Surface Radioiodination and Immunoprecipitation. After ¹²⁵I labeling of >98% viable cells with IODO-GEN (Pierce Chemical Co., Rockford, IL), ~5 × 10⁶ cells were incubated with appropriate dilutions of specific or control antibodies, washed, incubated with 0.7 mM of the crosslinking agent Dithiobis (succinimidyl propionate) (DSP), re-washed, and lysed with 1% NP-40 (18). Labeled proteins bound by antibody were separated by centrifugation using protein A-Sepharose (Zymed Laboratories, San Francisco, CA) for analysis by SDS-PAGE and autoradiography. In some experiments, DSP was omitted.

Results and Discussion

Polyclonal anti-hsp58 and anti-P1 stained PEER (TCR-γ/δ) and a second human TCR-γ/δ line established from immature thymocytes, but did not stain E6-1 (TCR-α/β), resting peripheral T cells, three T cell lines lacking TCRs (Fig. 1), or three nonlymphoid cell lines, HL60, THP-1, and U937 (data not shown). Staining with these antisera was specific, i.e., it did not involve binding of IgGs to Fcγ receptors, since indirect immunofluorescence with rabbit antibody to an irrelevant antigen, BSA, or with control IgGs.

Figure 2. Identification of a ~77-kD surface membrane molecule on γ/δ cells that reacts with anti-hsp58. (A) Immunoblots prepared with E6-1 whole cell lysate (lanes 1–3), plasma membranes enriched from homogenates of E6-1 (α/β) (lane 4) or PEER (γ/δ) lane (5), and PEER whole cell lysate (lane 6) were stained with antibodies to grp78 (lane 1), hsp70 (lane 2), P1 (lane 3), or hsp58 (lanes 4–6). (B) Immunoprecipitates of ¹²⁵I-labeled PEER cells were analyzed by SDS-PAGE and autoradiography (lane 1, anti-hsp58 plus 0.7 mM DSP, a crosslinker; lane 2, blank; lane 3, anti-hsp58 without DSP; lane 4, normal rabbit serum plus 0.7 mM DSP). Artificial internal labeling was excluded by the absence of the 58-kD band so prominent in the Western blots.

Figure 1. Surface staining of γ/δ cells by antibodies to hsp58/P1, the human homologue of the E. coli stress protein groEL. Ultracentrifuged anti-hsp58 and 10-fold higher concentrations of normal rabbit antiserum and/or rabbit anti-BSA antiserum were used as controls for specificity of staining. Cell included: (a and b) PEER (γδ); (c) human thymocyte line (γδ); (d and e) E6-1 (α/β); (f) MOLT-4 (TCR-); (g) peripheral blood T cells (primarily α/β); (h and i) JRT3/T3.5 (TCR-).
including normal rabbit IgG at concentrations up to 10-fold higher than that of anti-hsp58, was consistently negative. To further establish that reactivity with anti-hsp58 and anti-P1 was due to specific antibody binding, the above analyses were repeated with an independent assay, complement-dependent microcytotoxicity. The results paralleled those observed by indirect immunofluorescence: anti-hsp58 and anti-P1 killed 40–50% of γ/δ cells; cytotoxicity for peripheral T cells, α/β cell lines, and TCR− cell lines was 15% or less (control values). None of the cells were reactive with N27 or other antibodies to various members of the hsp70 family of stress proteins. Because expression of mycobacterial hsp65-related determinants can be induced by heat shock or other stimuli in mouse bone marrow macrophages (19), the indirect immunofluorescence experiments were repeated after subjecting the cells to brief heat shock. In no case was a difference in staining detected. Additional research will be required to determine whether other stimuli can induce anti-hsp58 reactivity on α/β T cells and to define the full range of expression of hsp58-related determinants on human lymphoid and nonlymphoid cells.

In biochemical studies, anti-hsp58 stained a ~77-kD protein in blots of PEER plasma membranes (Fig. 2A) and immunoprecipitated a single ~77-kD protein from 125I-labeled PEER cells (Fig. 2B). Anti-hsp58 reactivity with this surface molecule was not observed with E6-1, and was distinct from a major intracellular 58-kD protein in both PEER and E6-1, which presumably represents the mitochondrial hsp58/P1 reported previously (9, 20). Several additional bands of unknown significance that also were stained with anti-hsp58 in blots of PEER plasma membranes were not evident in the immunoprecipitation experiments, suggesting that the ~77-kD protein was the surface antigen target of interest. The ~77-kD band did not comigrate with known heat shock or glucose-regulated proteins of this approximate Mr. Nor did any of the panel of antibodies to members of the hsp70 family immunoprecipitate 125I-labeled proteins (data not shown). Conversely, the anti-hsp58 antiserum exhibited no reactivity with known stress proteins other than hsp58.

Taken together, these data suggest that constitutive surface expression of an hsp58-related ~77-kD protein may be a general property of lymphocytes bearing TCR-γ/δ and provide evidence for the existence of an hsp58-related protein(s) in cell compartments other than the mitochondrion. It remains to be determined whether this protein is a glycosylated version of mitochondrial hsp58 or, indeed, even a stress-inducible protein. In view of the recent discovery of several new groEL-related mammalian genes in addition to, and distinct from, that encoding mitochondrial hsp58 (R. A. Young, Whitehead Institute, Cambridge, MA, personal communication), we hypothesize that it is a product of one of these novel stress protein genes. Although the function and significance of this ~77-kD surface protein on γ/δ cells is only speculative at the present time, our data support previous evidence (1, 3–8) for the concept of a special relationship between groEL stress protein homologues and γ/δ cells.

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