

The Relationship of Aplastic Anemia and PNH

Neal S. Young, Jaroslaw P. Maciejewski, Elaine Sloand, Guiben Chen,
Weihua Zeng, Antonio Risitano, and Akira Miyazato

*Hematology Branch, National Heart, Lung, and Blood Institute,
Bethesda MD, USA*

Abstract

Bone marrow failure has been regarded as one of the triad of clinical manifestations of paroxysmal nocturnal hemoglobinuria (PNH), and PNH in turn has been described as a late clonal disease evolving in patients recovering from aplastic anemia. Better understanding of the pathophysiology of both diseases and improved tests for cell surface glycosylphosphatidylinositol (GPI)-linked proteins has radically altered this view. Flow cytometry of granulocytes shows evidence of an expanded PNH clone in a large proportion of marrow failure patients at the time of presentation: in our large NIH series, about 1/3 of over 200 aplastic anemia cases and almost 20% of more than 100 myelodysplasia cases. Clonal PNH expansion (rather than bone marrow failure) is strongly linked to the histocompatibility antigen HLA-DR2 in all clinical varieties of the disease, suggesting an immune component to its pathophysiology. An extrinsic mechanism of clonal expansion is also more consistent with knock-out mouse models and culture experiments with primary cells and cell lines, which have failed to demonstrate an intrinsic proliferative advantage for PNH cells. DNA chip analysis of multiple paired normal and PIG-A mutant cell lines and lymphoblastoid cells do not show any consistent differences in levels of gene expression. In aplastic anemia/PNH there is surprisingly limited utilization of the V-beta chain of the T cell receptor, and patients' dominant T cell clones, which are functionally inhibitory of autologous hematopoiesis, use identical CDR3 regions for antigen binding. Phenotypically normal cells from PNH patients proliferate more poorly in culture than do the same patient's PNH cells, and the normal cells are damaged as a result of apoptosis and overexpress Fas. Differences in protein degradation might play a dual role in pathophysiology, as GPI-linked proteins lacking an anchor would be predicted to be processed by the proteasome machinery and displayed in a class I H.A. context, in contrast to the normal pathway of cell surface membrane recycling, lysosomal degradation, and presentation by class II HLA. The strong relationship between a chronic, organ-specific immune destructive process and the expansion of a single mutant stem cell clone remains frustratingly enigmatic but likely to be the result of interesting biologic processes, with mechanisms that potentially can be extended to the role of inflammation in producing premalignant syndromes.

1. Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) came to medical attention because of its unusual symptom of pigment loss in the urine [1]. "Intermittent hematuria" was later recognized as due to the destruction of red cells in the circulation, and the unusual intravascular hemolysis shown to be secondary to the susceptibility of red cells to a non-antibody component of perturbed serum preparations. When in vitro hemolysis on expo-

sure to acidified serum was utilized to formulate the diagnostic Ham test, the clinical signs of the disease were broadened and PNH was more fully characterized by a classic triad of hemolysis, venous thrombosis, and marrow failure. Indeed, thrombotic disease, which is often in unusual anatomical locations, recurrent, and resistant to therapy, is the major cause of death among Western patients. In contrast, pancytopenia predominates as a manifestation of PNH in Asian countries. PNH is closely related to the disease aplastic anemia, in which

immune-mediated hematopoietic cell destruction results in empty bone marrow [2]: historically, patients with aplastic anemia have appeared to evolve to PNH, with evidence of hemolysis combined with a positive Ham test appearing months or years after successful treatment with immunosuppressive drugs [2].

The somatic genetic defect in PNH and its biochemical consequence of defective presentation of proteins linked to the cell surface by a glycosylphosphatidylinositol (GPI) anchor have now been fully characterized. Historically, the mechanism of red cell lysis by treated sera was known to be activation of the alternative pathway of complement [3]; the erythrocyte defect was identified functionally as an inability to inactivate complement on the cell surface, in turn due to a deficit in specific membrane proteins (decay accelerating factor [DAF or CD55] and membrane inhibitor of reactive lysis [MIRL or CD59]). It was puzzling that not only these proteins but a wide variety of others, including leucocyte alkaline phosphatase and erythrocyte acetylcholinesterase, were absent from the PNH cell surface. Ultimately, demonstration of the enzymatic release of alkaline phosphatase by a specific phospholipase led to the identification of the common structural feature of a highly diverse group of missing proteins—a distinctive biochemical linkage to the cell membrane, the (GPI) anchor. Preformed GPI is attached covalently to proteins that bear the appropriate carboxyl sequence and links them to the plasma membrane through the phosphatidylinositol moiety. GPI-anchored proteins also appear to cluster on the cell surface in biophysically distinctive, detergent-insoluble regions, termed rafts. There are almost 100 different GPI-anchored mammalian cell proteins, including enzymes, adhesion molecules, receptors, and blood group antigens [4]. The GPI anchor is evolutionarily conserved, and most parasite proteins use this linkage [5], and the biosynthesis of the GPI anchor was determined first for trypanosomes and later in deficient mammalian cell lines [6]. The genetic defect in PNH, discovered by expression cloning and then phenotypic correction of mutant cell lines, was localized to the *PIG-A* (phosphatidylinositol glycan-class A) gene [7]; *PIG-A* encodes a protein required for the transfer of N-acetylglucosamine to phosphatidylinositol, an early step in the synthesis of the anchor [3]. Immortalized cell lines from PNH patients all belong to the class A complementation group; the most frequent *PIG-A* alterations are small deletions that create stop codons or frameshifts, followed by missense mutations and small insertions.

PNH is a hematologic disease, and to date *PIG-A* gene mutations have only been described in hematopoietic cells. The cellular clonal origin of PNH was first inferred from G6PD enzyme analysis of circulating red cells in informative heterozygous females, and by implication all defective erythrocytes, leucocytes, and platelets originated from a single hematopoietic progenitor [8]. Indeed, in PNH patients GPI-anchored proteins are missing from variable proportions of erythrocytes, granulocytes, platelets, and some lymphocytes,

consistent with the activity of an aberrant hematopoietic stem cell clone. Because of the X chromosome location of *PIG-A*, a mutation is dominant at the cellular level due to Lyonization in women and hemizyosity in men.

2. PNH/Aplastic Anemia

Analysis of GPI-anchored proteins by flow cytometry is both more accurate and sensitive than the Ham test, and examination of granulocytes allows study of even heavily transfused patients. From its first application in aplastic anemia [9], flow cytometry has revealed an expanded PNH clone in a very high proportion of patients; in the most recent analysis of our large series of over 200 cases, PNH was present in almost half [10]. Expansion of GPI-anchored protein-negative cells is almost always apparent at the time of diagnosis of aplastic anemia, and thus PNH is only unusually a “late” clonal event, as was incorrectly inferred from Ham test results. Indeed, about the same number of patients acquire a clone as showed spontaneous disappearance of aberrant cells. Furthermore, PNH clonal expansion is also associated with the myelodysplastic syndromes: present in 18% of 120 of our NIH patients (mainly referred at early FAB stages for the diagnosis and treatment of pancytopenia, rather than progression to leukemia) [10].

Flow cytometric testing indicates that marrow failure and PNH clonal expansion frequently coexist. Functional studies also show similarity between these syndromes. Aplastic anemia is characterized by a markedly diminished proliferative capacity of the marrow, as assayed in a variety of in vitro culture systems, including assays in semisolid medium for myeloid (CFU-GM), erythroid (BFU-E, CFU-E), megakaryocytic (CFUMeg), and multipotential (CFUGEMM) progenitor cells; primitive progenitors (LTC-IC), and CD34 cell growth in long term bone marrow cultures. Although the morphology of the marrow in PNH is often relatively cellular, especially demonstrating erythroid hyperplasia, results in colony assays are similar to those observed in overt aplastic anemia [11-13]. Thus patients with PNH have evidence of a functional defect in blood cell production with other marrow failure syndromes.

3. Pathophysiology of PNH Clonal Expansion

Can the genetics of *PIG-A* explain the pathophysiology of PNH? Does acquisition of the somatic mutation inexorably lead to clonal expansion? In the simplest model, *PIG-A* deficiency would confer on mutant cells an intrinsic growth or survival advantage. Clinical observations and most experimental data do not support this hypothesis. PNH as a disease does not behave like a leukemia and in most patients the proportion of PNH cells is stable over years and their differentiation proceeds along the usual lineage pathways. PNH is a rare disease, but normal individuals harbor tiny PNH clones: very small numbers, 20-60/million, of GPI-anchored protein-deficient, *PIG-A*- granulocytes were detected after re-

peated cell sorting of blood of healthy volunteers [14]. In chimeric knock-out mice, *PIG-A*⁻ cells also constitute a minor, static fraction of the marrow or circulating hematopoietic compartments [15-17]. *PIG-A*⁻ and normal cells in general behave similarly in paired tissue culture assays. One group reported a major difference between *PIG-A*⁻ cells and controls in susceptibility to apoptosis [18], but this finding has not been reproduced in other laboratories [19] (including our unpublished data; granulocytes from patients with a variety of marrow failure syndromes, including PNH, have been described as globally resistant to apoptosis [20].) Additionally, there is no known molecular mechanism for GPI-anchored proteins to influence apoptotic cell death. Marrow cells from some PNH patients showed superior survival in SCID mice [20], although the normal cells in these experiments showed unusually poor persistence, and the mice retained some immunological function. In our recent analysis of the transcriptome of paired normal and PNH cells, including cell lines (K562, Ramos) and immortalized lymphoblastoid cells derived from patients, using DNA chip technology to detect differences in gene expression, there were remarkably few differences (our unpublished data). These results suggest that the *PIG-A* mutation is not associated with major changes in the cell's program for growth, differentiation, or death [21].

In a second model, the *PIG-A* defect must be combined with some extrinsic factor to promote clonal expansion. Patients often harbor multiple clones with different *PIG-A* mutations [22], consistent with their emergence under selective pressure. Lymphocytes of PNH phenotype and *PIG-A* genotype appeared in lymphoma patients treated with Campath-1, a monoclonal antibody that coincidentally recognizes a GPI-anchored protein [21]. The frequent association of PNH with aplastic anemia first suggested that the hypocellular marrow milieu might be such a permissive environment [23;24]. However, while PNH clones are present in a very large proportion of aplastic anemia patients, PNH does not occur after chemotherapy or radiation-induced aplasia, following stem cell transplant, or in constitutional Fanconi anemia [25]. The presence of PNH does appear to correlate in particular with immunologically mediated marrow failure, and in our experience serves as a positive predictor of response to immunosuppressive therapy in aplastic anemia and myelodysplasia [26]. Furthermore, the presence of PNH clonal expansion, not aplastic anemia, is strongly associated with the HLA-DR2 class II histocompatibility locus [26].

In most patients with acquired aplastic anemia, the underlying pathophysiology appears to be immune-mediated [27]. In vitro inhibition of hematopoiesis by lymphocytes or T cells was an early clue to this mechanism [28]; many of the patients tested probably also had expanded PNH clones, and T cell suppression of erythroid cultures also has been described for one PNH patient without marrow aplasia [29]. Patients with aplastic anemia frequently show evidence of lymphocyte activation, especially of cytotoxic T cells, which over-

express type I cytokines like interferon- and tumor necrosis factor which in turn induce Fas-mediated apoptosis in hematopoietic target cells [27]. While the antigens that trigger autoimmunity are unknown, fine analysis of the T cell receptor repertoire has suggested that the immune response is indeed antigen driven in both aplastic anemia [30] and PNH [31]. In our recent study of patients with PNH/aplasia, we found not only surprisingly limited utilization of the V beta chain of the T cell receptor, but that among different patients' the dominant (CD4) T cell clones used an identical CDR3 region for antigen binding; this CDR3 was first isolated from one patient's marrow and shown to specifically inhibit autologous hematopoiesis [32].

One possible mechanism for clonal expansion in PNH is escape from immune surveillance, as a number of mammalian GPI-anchored proteins are ligands for T cell receptors, including CD58 and CD59 (for CD2), or serve immune functions, as do CD14 and the Fc III receptor; the absence of such a molecule might result in a global failure of PNH cells to be recognized by the immune system (immune escape is a proposed role of protozoan GPI-anchored proteins like variable surface glycoprotein of trypanosomes). For one inhibitory T cell clone derived from an aplastic anemia patient, anti-CD59 antibody appeared to block cytotoxicity against lymphoblastoid cell targets [33]. We described a GPI-anchor protein-deficient Ramos cell line that was extremely resistant to natural killer cell lysis, but the *PIG-A* gene in these cells was normal [34]. Recently, an interesting mechanism was described for proinflammatory cytokine production after heat shock protein stimulation through CD14 [35]. Nevertheless, extensive efforts in several laboratories to determine general differences between normal and *PIG-A*⁻ target cells in a wide variety of immunologic assays have been negative [36] (and others largely unreported). Finally, most clinical data do not show changes that might be predicted in either the presence or size of the PNH clone, before and after immunosuppressive therapy, or on hematologic recovery and at recurrence of pancytopenia; in most cases, the proportion of GPI-anchored protein-deficient cells remains relatively constant over time [10].

In recently published work a major difference was observed in cell culture of PNH patients' marrows, but as a deficiency among the "normal" cells rather than an advantage for the *PIG-A* mutant cells: CD34 cells, separated based on the presence or absence of CD59, were cultivated in the presence of growth factors; the cells of PNH phenotype behaved normally, producing several hundred thousand progeny, while CD34 cells of normal phenotype (but from the PNH patients) showed little growth. An inference from these results was that the PNH cells had survived an in vivo injury sustained by the normal progenitors. That this insult might indeed be immunologic was suggested by the expression of Fas on the normal but not on the GPI-anchor protein deficient cells and concomitant sensitivity to Fas-ligand mediated apoptosis [37]. We have reproduced these results in a larger number of patients: compared to CD34 cells

of the PNH phenotype, normal cells from the patients' bone marrow show lower cloning efficiency in semisolid medium, increased CD95 expression, and frank evidence of apoptosis mainly in the cells of normal phenotype as determined by annexin V and caspase 3 staining [38]. Longer term culture of these marrow specimens suggest that once dying cells have progressed through apoptosis, the remaining viable cells of normal and PNH phenotype have similar growth and survival characteristics—consistent with selective injury *in vivo* rather than an intrinsic cellular difference.

Nevertheless, the character of the interaction of a *PIG-A* clone and either the normal hematopoietic environment or the failed marrow milieu and indeed of the cause and effect sequence of PNH and aplastic anemia remains frustratingly enigmatic. A general deficit in effector recognition of a defective cellular target, as for example due to decreased binding of costimulatory molecules secondary to the GPI-anchor protein deficiency, would explain the emergence of normally quiescent but now resistant PNH clones under conditions of autoimmune attack. However, most experiments to directly test this possibility have been negative; nevertheless, the effect could be subtle and only manifest under special *in vivo* circumstances. Also, dependence of PNH cell expansion on the presence of immune mediators is hard to reconcile with the stability of PNH clones over time and despite changes in blood counts and treatment. Alternatively, the mutated PNH cell might have a more primary role in the development of bone marrow failure. GPI-anchored proteins might serve as antigens, either due to molecular mimicry with exogenous peptides or subsequently involved as part of the process of antigenic spread. Finally, the PNH clone itself might induce autoimmunity. One possibility that we are currently investigating is the consequence of a global alteration in protein trafficking due to absence of GPI anchors. Essentially, what is the fate of GPI-anchorless proteins in the PNH cell? Under normal circumstances, membrane-bound protein degradation should occur largely in lysosomes, leading to presentation of peptides in the context of class II HLA. In contrast, GPI-anchorless proteins in the *PIG-A* cell should be processed by the proteasome and their peptides displayed via class I molecules. Published experiments indicate that for chimeric genes transfected into cell lines, constructs with defective carboxyl termini produced proteins which were retained in the endoplasmic reticulum [39] and degraded via the proteasome [40]. A model in which protein trafficking and degradation were primary to the pathophysiology of PNH would provide a role for both GPI-anchored protein antigenicity (increased or novel peptide presentation by class I MHC) and a mechanism for escape (decreased class II display). There may be other major, unanticipated consequences of failed GPI anchor synthesis beyond deficient expression of certain membrane proteins.

In PNH, models and experiments will need to account for a number of remaining puzzling questions, both clinically relevant and of general biologic importance.

Biochemically, little is understood of the presumed special function of the GPI-anchor in the mammalian cell: in altering the mobility of membrane proteins, in internal signal transduction, in collaboration with other proteins in rafts, and in cell-cell protein transfer, and even for shedding from the cell surface (there is sufficient inactive phospholipase C in plasma to release virtually all GPI-anchor proteins from circulating cells). For stem cell biology, why is there such a tiny proportion of *PIG-A* progeny in normal individuals; why do *PIG-A* clones persist for years, even decades, supplying the entire blood production of a patient; and why do clones appear to extinguish entirely in rare individuals? Clinically, we need to account not only for the close relationship of a clonal, somatically acquired genetic disorder and immunologically mediated bone marrow failure, but also for evidence of generalized marrow depression in all forms of PNH, the multiplicity of clones in disease, and the progression to refractory fatal thrombosis or aplastic anemia in many patients.

References

1. Rosse W. A Brief History of PNH. In PNH and the GPI-linked Proteins, ed. NS Young, J Moss. 1-20. Academic Press, San Diego, CA., 2000.
2. Frickhofen N, Rosenfeld SJ. Immunosuppressive treatment of aplastic anemia with antithymocyte globulin and cyclosporine. *Semin Hematol.* 2000;37:56-68.
3. Luzzatto L, Nafa K. Genetics of PNH. In PNH and the GPI-linked Proteins, ed. NS Young, J Moss. 21-48. Academic Press, San Diego, CA., 2000.
4. Okazaki I, Moss J. The Function of GPI-Anchored Proteins. In PNH and the GPI-linked Proteins, ed. NS Young, J Moss. 159-177. Academic Press, San Diego, CA., 2000.
5. Schofield, L. GPI in Lower Animals. In PNH and the GPI-linked Proteins, ed. NS Young, J Moss. 179-198. Academic Press, San Diego, CA., 2000.
6. Seveler D, Chen R, Medof ME. Synthesis of the GPI Anchor. In PNH and the GPI-linked Proteins, ed. NS Young, J Moss. 199-220. Academic Press, San Diego, CA., 2000.
7. Takeda J, Miyata T, Kawagoe K, et al. Deficiency of the GPI anchor caused by a somatic mutation of the *PIG-A* gene in paroxysmal nocturnal hemoglobinuria. *Cell.* 1993;73:703-711.
8. Oni SB, Osunkoya BO, Luzzatto L. Paroxysmal nocturnal hemoglobinuria: evidence for monoclonal origin of abnormal red cells. *Blood.* 1970;36:145-152.
9. Schrenzenmeier H, Hertenstein B, Wagner B, Raghavachar A, Heimpel H. A pathogenetic link between aplastic anemia and paroxysmal nocturnal hemoglobinuria is suggested by a high frequency of aplastic anemia with a deficiency of phosphatidylinositol glycan proteins. *Exp Hematol.* 1995;23:81-87.
10. Maciejewski JP, Rivera C, Dunn D, Young NS. Clinical features of the relationship between aplastic anemia and paroxysmal nocturnal hemoglobinuria. *Brit J Haem.* In Press., 2001.
11. Rotoli B, Robledo R, Luzzatto L. Decreased number of circulating BFU-Es in paroxysmal nocturnal hemoglobinuria. *Blood.* 1982;60:157-159.
12. Moore JG, Humphries RK, Frank MM, Young N. Characterization of the hematopoietic defect in paroxysmal nocturnal hemoglobinuria. *Exp Hematol.* 1986;14:222-229.
13. Issaragrisil S, Piankijagum A, Chinprasertsuk S, Kruatrachue M. Growth of mixed erythroid-granulocytic colonies in culture derived from bone marrow of patients with paroxysmal nocturnal hemoglobinuria without addition of exogenous stimulator. *Exp Hematol.* 1986;14:861-866.

14. Araten DJ, Nafa K, Pakdeesuwana K, Luzzatto L. Clonal populations of hematopoietic cells with paroxysmal nocturnal hemoglobinuria genotype and phenotype are present in normal individuals. *Proc Natl Acad Sci USA*. 1999;96:5209-5214.
15. Kinoshita T, Bessler M, Takeda J. Animal Models of PNH. In PNH and the GPI-linked Proteins, ed. NS Young, J Moss. 139-158. Academic Press, San Diego, CA., 2000.
16. Dunn D, Yu J, Nagarjan S, et al. A knock-out model of paroxysmal nocturnal hemoglobinuria: PIG-A- hematopoiesis is reconstituted following intercellular transfer of GPI-anchored proteins. *Proc Natl Acad Sci USA*. 1996;93:7938-7943.
17. Kawagoe K, Kitamura D, Okabe M, et al. Glycosylphosphatidylinositol-anchor-deficient mice: implications for clonal dominance of mutant cells in paroxysmal nocturnal hemoglobinuria. *Blood*. 1996;87:3600-3606.
18. Brodsky RA, Vala MS, Barber JP, Jones RJ, Medof ME. Resistance to apoptosis caused by PIG-A gene mutations paroxysmal nocturnal hemoglobinuria. *Proc Natl Acad Sci USA*. 1997;94:8756-8760.
19. Ware RE, Nishimura J, Moody MA, Smith C, Rosse WF, Howard TA. The PIG-A mutation and absence of glycosylphosphatidylinositol-linked proteins do not confer resistance to apoptosis in paroxysmal nocturnal hemoglobinuria. *Blood*. 1998;92:2541-2550.
20. Iwamoto N, Kawaguchi T, Horikawa K, et al. Preferential hematopoiesis by paroxysmal nocturnal hemoglobinuria clone engrafted in SCID mice. *Blood*. 1996;87:4944-4948.
21. Hertenstein B, Wagner B, Bunjes D, et al. Emergence of CD52-, phosphatidylinositolglycan-anchor-deficient T lymphocytes after in vivo application of Campath-1H for refractory B-cell non-Hodgkin lymphoma. *Blood*. 1995;86:1487-1492.
22. Bessler M, Mason PJ, Hillmen P, et al. Paroxysmal nocturnal haemoglobinuria (PNH) is caused by somatic mutations in the PIG-A gene. *EMBO J*. 1994;13:110-7.
23. Young NS. The problem of clonality in aplastic anemia. Dr. Dameshek's riddle, restated. *Blood*. 1992;79:1385-92.
24. Luzzatto L, Bessler M, Rotoli B. Somatic mutations in paroxysmal nocturnal hemoglobinuria: a blessing in disguise. *Cell*. 1997;88:1-4.
25. Dunn DE, Tannawattanacharoen P, Bocconi P, et al. Paroxysmal nocturnal hemoglobinuria cells in patients with bone marrow failure syndromes. *Ann Intern Med*. 1999;131:401-408.
26. Maciejewski JP, Follman D, Rivera CE, Brown K, Simonis T, Young NS. Increased frequency of HLA-DR2 in patients with paroxysmal nocturnal hemoglobinuria and PNH/aplastic anemia syndrome. *Blood*. In Press., 2001.
27. Young NS, Maciejewski J. The pathophysiology of acquired aplastic anemia. *N Engl J Med*. 1997;336:1365-1372.
28. Young NS. Pathophysiology II: immune suppression of hematopoiesis. In *Aplastic Anemia, Acquired and Inherited*, ed. NS Young, BP Alter. 68-99. W.B. Saunders, Philadelphia., 1994.
29. Merchav S, Tatarsky I, Chezar J, Sharon R, Rosenbaum H, Schechter Y. Paroxysmal nocturnal hemoglobinuria associated with in vitro inhibition of erythropoiesis by bone marrow T lymphocytes. *IMAJ*. 2000;2:22-24.
30. Zeng W, Nakao S, Takamatsu H, et al. Characterization of T-cell repertoire of the bone marrow in immune-mediated aplastic anemia: Evidence for the involvement of antigen-driven T-cell response in cyclosporine-dependent aplastic anemia. *Blood*. 1999;93:3008-3016.
31. Karadimitris A, Manavian JS, Thaler HT, et al. Abnormal T-cell repertoire is consistent with immune process underlying the pathogenesis of paroxysmal nocturnal hemoglobinuria. *Blood*. 2000;96:2613-2620.
32. Zeng W, Maciejewski JP, Chen G, Young NS. Limited heterogeneity of T-cell receptor VB usage in aplastic anemia. *Journal of Clinical Investigation*. In Press., 2001.
33. Takami A, Zeng W, Wang H, Matsuda T, Nakao S. Cytotoxicity against lymphoblastoid cells mediated by a T-cell clone from an aplastic anemia patient: role of CD59 on target cells. *Br J Haematol*. 1999;107:791-796.
34. Dunn DE, Nagakura S, Tanawattanacharoen P, Knez J, Medof ME, Young NS. The PNH phenotype (GPI-anchored protein-deficiency) protects hematopoietic targets against lymphocytotoxic attack [abstract]. *Blood*. 1997;90(Suppl 1):407a.
35. Asea A, Kraeft SK, Kurt-Jones EA, et al. HSP70 stimulates cytokine production through a CD14-dependent pathway, demonstrating its dual role as a chaperone and cytokine. *Nat Med*. 2000;6:435-442.
36. Hollander N, Selvaraj P, Springer TA. Biosynthesis and function of LFA-3 in human mutant cells deficient in phosphatidylinositol-anchored proteins. *J Immunol*. 1988;141:4283-4290.
37. Chen R, Nagarajan S, Prince GM, et al. Impaired growth and elevated FAS receptor expression in PIGA(+) stem cells in primary paroxysmal nocturnal hemoglobinuria. *J Clin Invest*. 2001;106:689-696.
38. Chen G, Kirby M, Zeng W, Young NS, Maciejewski JP. Superior growth of glycosylphosphatidylinositol-anchored protein-deficient progenitor cells in vitro is due to the higher apoptotic rate of progenitors with normal phenotype in vivo. *Exp. Hematol.*, in press.
39. Fields MC, Morani P, Li W, Keller G-A, Caras IW. Retention and degradation of proteins containing an uncleaved glycosylphosphatidylinositol signal. *J Biol Chem*. 1994;269:10830-10837.
40. Oda K, Ikehara Y, Omura S. Lactacystin, an inhibitor of the proteasome, blocks the degradation of a mutant precursor of glycosylphosphatidylinositol-linked protein in a pre-Golgi compartment. *Biochem Biophys Res Commun*. 1996;219:800-805.