He reshaped the forefront of xenotransplantation:

Agustin Pasqual Dalmasso (1933-2021)

Jeffrey L. Platt[a] and Gregory M. Vercellotti[b]

Departments of Surgery and Microbiology & Immunology, University of Michigan, Ann Arbor, Michigan USA and Department of Medicine, Division of Hematology, Oncology and Transplantation, University of Minnesota Medical School, Minneapolis, Minnesota USA

a. Departments of Surgery and Microbiology & Immunology, University of Michigan, Ann Arbor, Michigan USA
b. Department of Medicine, Division of Hematology, Oncology and Transplantation, University of Minnesota Medical School, Minneapolis, Minnesota USA

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Address communications to:
Jeffrey L. Platt, M.D.
Transplantation Biology
A520B MSRB I
1150 W. Medical Center Drive, SPC 5656

Ann Arbor, Michigan 48109-5656
734 615-7755 telephone
734 615-7141 fax
plattjl@umich.edu

Agustin Pasqual Dalmasso died in December 2021. He was 88 years old and an Honorary Member of the International Xenotransplantation Association. Gus[1] made seminal contributions to understanding and overcoming the barrier complement system poses to xenotransplantation. Those endeavoring to advance xenotransplantation to clinical application and those seeking the most topics in which to devote their life's work could do no better than examining how Gus approached the subjects of his life's work.

The forefront of xenotransplantation from the 1990s to the present

The subject of Gus Dalmasso's work most familiar to those working to the field of xenotransplantation concerned an hypothesis he put forward in 1990 that incompatibility between the complement system of a xenograft recipient and complement regulatory proteins expressed in a xenograft (i.e. homologous restriction) might underlie the extraordinary susceptibility of xenografts to immediate destruction **(1)**. Gus offered this hypothesis as an explanation for the dramatic difference between the outcomes of ABO-incompatible allografts and porcine organ xenografts in non-human primates. Recipients of both grafts had natural antibodies that could bind to graft endothelium and activate complement but the allografts only occasionally suffered hyperacute acute rejection while the xenografts always did so. Furthermore, temporary removal of natural antibodies from recipients of ABO-incompatible kidney transplants enabled the kidneys to survive and function nearly as well as ABO-compatible kidney transplants whereas porcine organs transplanted into non-human primates at that time never survived longer than days to a few weeks.

Gus tested this idea by isolating decay accelerating factor (CD55) from human erythrocytes, introducing the glycosyl phosphatidylinositol anchored human protein into cell membranes of porcine endothelial cells, and testing whether and to which extent the human protein protected the porcine cells from lysis by human complement **(2)**. The isolated and incorporated human CD55 conferred dose-dependent protection and the work provided the first experimental justification for genetic engineering of pigs as potential sources of xenografts.

The contribution of incompatibility in regulation of complement to the barrier to xenogeneic organ transplantation is no longer an hypothesis; It is fundamental to transplantation immunology and the archetype of a burgeoning set of incompatibilities that potentially influence transplantation.

Likewise, the application of genetic engineering to address such incompatibilities is no longer a proposition but likely the first approach to be considered in efforts to improve outcomes of transplants. Yet, anyone endeavoring to improve the outcome of xenografts and anyone who might wonder why the outcomes of xenografts and anyone thinking of engineering still better porcine sources of xenografts might be well served by reviewing how Gus weighed decay accelerating factor against other complement regulators in suggesting how the problem might be solved **(2)**.

The forefront of molecular immunology in the mid 1960s: biochemistry and immunobiology of complement at Scripps Research Institute

Anyone wishing to understand how organs from pigs engineered to overcome incompatibility of complement regulation and eliminate to saccharide targets of natural antibodies might still be subject to complement-mediated injury might benefit from exploring Gus Dalmasso's first investigation of conditions that facilitate activation of C3 **(3)**, the pivotal and most abundant component of complement. When Gus undertook this work with Hans Muller-Eberhard in 1963 **(4)**, complement was long considered the critical effector of humoral immunity, and particularly of hemolysis, and components of complement had been isolated, partly characterized and quantitated in plasma **(5)**. But how complement proteins interacted to generate effector functions was incompletely understood despite 50 years of robust debate **(5, 6)**.

The first project Gus undertook at Scripps, and one potentially pertinent for xenotransplantation today, concerned the mechanisms through which C4 and C3 interact with cell membranes **(3)**. Then, in the early 1960s, complement interactions were believed to proceed stepwise, beginning with the binding of antibody to a cell surface antigen (**Figure 1)**. Although antibody was generally believed to trigger complement reactions, many thought the preponderance of steps, and especially steps involving C4 and C3, occurred independent of bound antibody. Gus therefore wanted to develop a system in which C4 and/or C3 could be activate in the absence of antibodies. Development of such a system was challenging from both a technical and a political perspective. Ten years earlier, Louis Pillemer claimed to discover that serum protein, properdin, together with microbial polysaccharide could activate complement independent of antibody or involvement of C1, C4 and C2 **(7).** But, Pillemer's claim was vociferously disputed by claims that properdin preparations were contaminated by natural antibodies and that these antibodies together with C1, C4 and C2 had activated complement **(8)**. Thus, when Gus began to investigate complement, his new colleagues at Scripps and most others thought binding of antibodies was the essential first step in the interaction of complement with cells, as **Figure 1** depicts **(5, 9)**. Given popular belief about the mechanism of complement activation and recent controversy, Gus's effort to investigate activation of complement in the absence of antibodies was certainly a courageous endeavor.

To investigate the activation of complement independent of antibodies, Gus used human sera as sources of complement and autologous human erythrocytes as cellular targets. As expected, the

antibodies in the sera used did not bind to autologous erythrocytes, C3 did attach to cell membranes and lysis did not occur. However, if the sera were pre-incubated with polyethylene glycol (PEG) and if the autologous erythrocytes were pre-treated with trypsin or neuraminidase, C4 and C3 firmly attached to autologous cell membranes and complement-mediated lysis ensued.

Gus believed the lysis of autologous erythrocytes reflected two processes. As one process, addition of PEG to serum caused immunoglobulin to aggregate and the immunoglobulin aggregates triggered activation of complement. As a second process, treatment of cells with trypsin or neuraminidase allowed C4 and C3 to attach to cells thereby recruiting other complement proteins to cause lysis in the absence of bound immunoglobulin. Gus posited that trypsin and neuraminidase treatment rendered the erythrocytes more susceptible by removing negatively charged saccharides that would otherwise "repel" charged complement proteins.

The separation of the "site" of complement activation from cells lysed by complement, as Gus reported **(3)**, anticipated discovery that activation of complement by polynucleotides **(10)** or cobra venom factor **(11)** could likewise target bystander cells for lysis. Separation of complement-mediated lysis from requisite binding of antibodies also set the stage for rediscovery of the alternative pathway of complement activation during the ensuing decade **(11-14)**. Decades later polyethylene was found to activate the alternative pathway of complement **(15)**.

But we think the most interesting and important concept traceable to Gus's earliest work, concerns the implication that acidic cell surface moieties released by trypsin or neuraminidase might govern the specificity of complement reactions **(3)**. In the early 1960s, when antibody binding was considered essential for initiating activation of complement, the killing of foreign cells and microorganisms and sparing of autologous cells was explained by immune specificity and tolerance. Viewed from that perspective, complement mediated lysis of autologous cells in Gus's model **(3)** departed dramatically from the classical notion of *horror autotoxicus* **(16)** and clearly demanded an explanation. Gus speculated that trypsin (via cleavage of glycoproteins) and neuraminidase release negatively charged saccharides that otherwise would repel complement from cell surfaces and that antibody binding to foreign cells might counteract the negative charge on cell membranes, a mechanism that would later explain how certain antibodies, such as IgA, could directly activate the alternative pathway complement. This finding also presaged discovery neuraminic acid residues on erythrocytes recruit and promote activity of factor H, the critical regulator of the alternative pathway convertase **(17)**, and enable function of CD55 and CD59 **(18)**.

Nucleated cells such as endothelial cells are often eschewed as targets for assaying complement-mediated lysis because regulation of complement on nucleated cells increases the complexity of reactions representing the complement cascade and raises the threshold for lysis. But, the

complexity and hurdles to activation of complement on normal nucleated cells made Gus keen to elucidate the mechanism of complement activation on porcine endothelial cells **(19)** and the pathogenic functions generated by complement activation on endothelial cells **(20)**. The complexity of complement interactions with endothelial cells also sparked Gus's interest in determining whether and how the binding of antibodies per se or coupled with complement regulation might increase regulation of complement, further suppressing complement-mediated injury **(21, 22)**.

Because the mechanisms controlling activation of complement on endothelial cells depend in part on the expression of heparan sulfate proteoglycan on cell membranes and in extracellular matrices, Gus enthusiastically joined our investigation of heparan sulfate metabolism. Thus, he worked with us to explore how activation of complement on the surface or in the vicinity of endothelial cells (e.g. during ischemia) causes shedding of heparan sulfate thereby compromising regulation of complement and heightening susceptibility to injury and lysis **(23)**. We have since learned that metabolism of heparan sulfate and complement broadly impacts defenses against microbial and environmental challenges and that insight in turn inspired development of novel therapeutic agents that could find application in xenotransplantation.

The forefront of research in immunology in early 1960s: investigation of the thymus at the University of Minnesota

After completing education in medicine, Gus trained in immunology and laboratory medicine at the University of Minnesota. In the early 1960s, the program headed by Robert Good was a leading center for research in clinical and experimental immunology. Good and coworkers were then engaged in a highly contested race to discover how exactly thymus contributed to the development and functions of the immune system **(24-26).** The program also included leading work on complement and on transplantation. Gus sought to determine the thymus might contribute to various types of immune responses. At that time, the relatively scarce understanding of thymus function had been deduced almost entirely from the characteristics of immunodeficiency associated with removal of the thymus from newborn animals **(27, 28)**. However, results varied considerably between laboratories and between species and strains of animals used and technical aspects of procedures detracted further from consistency of observations.

Gus was positioned at the center of this rapidly moving and contentious field. His transition to work on complement and away from cell-mediated immunity and his wry skepticism would have made him the ideal individual to reflect on the era and the setting. But Gus was too modest to try to settle disputes between giants. Still, several observations Gus made could prove pertinent to xenotransplantation.

Soon after arrival in Minnesota Gus participated in experiments addressing whether and in what ways removal of the thymus soon after birth could lead to immunodeficiency of mature mice and rabbits **(28, 29)**. The experiments confirmed Miller's report **(27)** that removal of the thymus at birth from some mice of some strains caused immunodeficiency severe enough to impair ability of reject allografts but extended the observation to show that rabbits subjected to removal of thymus at birth exhibited unimpaired ability to reject allografts. Humans resemble rabbits in this regard and therefore must receive immunosuppression to maintain cardiac transplants, often accompanied by removal of the thymus, performed early in life.

The work did reveal that removal of the thymus at birth confers at least one defect relevant to cardiac allo- or xeno-transplantation. Removal of the thymus early in life caused long-term impairment in production of antibodies against partially purified bovine albumin in rabbits and against bacteriophage in rabbits and mice. Decades later our own work revealed that removal of the thymus in the newborn and in mature individuals impairs affinity maturation of T cell-dependent B cell responses and that affinity maturation, reflecting somatic hypermutation and selection, enables generation of broadly neutralizing antibodies to viruses. Although Gus conducted his research before thymus-dependent cells were defined and found to influence B cell responses, his results provide an early hint about limiting facets of immunity.

Concluding remarks

Gus Dalmasso quietly but profoundly impacted everyone he knew. His love for family and children, his passion for opera and Argentine soccer were central to his life and conferred untold benefit. He profoundly benefitted the many patients he served at the University of Minnesota and at Minneapolis Veterans Administration Medical Centers. The incisive and innovative thinking that fueled his research were translated into state-of-the-art practice of transfusion and laboratory medicine and consultation, both alloyed with caring and compassion. Gus provided exceptional guidance and mentorship to many students, residents, trainees and junior colleagues - giving of his time with surpassing generosity and always welcoming discussion of questions and ideas and collaboration.

During a career spanning five decades, Gus conducted research at the forefront of three fields. He and the teams he joined made preeminent advances in understanding the functions of the thymus, the chemistry and functions of complement and the barriers to xenotransplantation. We discussed a few of the contributions we think might interest those working today in the field of xenotransplantation.

Some might wonder how an unassertive and humble individual like Gus would so often arrive at the forefront of the subjects investigated. We imagine Gus would say that chance had brought him to

the right place at the right time. While we are second to none in our regard for good luck, we would respectfully disagree. Gus quietly but assuredly made the advances we described (among others) by conceiving and testing possibilities that countered canonical thinking. His idea concerning the pathogenic importance of regulation of complement in xenotransplantation emerged when antibodies and antibody-specificity where considered the key hurdle to success of xenotransplantation. It was his idea that raised interest in application of genetic engineering in xenotransplantation and led to the forefront of the field today.

The research of the most widely recognized, vociferous leaders can follow erroneous paths if colleagues and student are reluctant to offer critical feedback. Above we discussed how those investigating complement were misled for a decade or more about antibody-independent pathways for triggering complement reactions and how close Gus came to correcting that error. It is possible Gus came so close by chance, but we suspect Gus's natural skepticism and aversion to dogma enabled him to pursue antibody-independent recruitment of complement when he did.

Gus was not one to seek or demand credit for his work. We imagine think he might well have resisted efforts to connect so much of his work with current forefronts. But we must close by commending the membership and leaders of the International Xenotransplantation Association for having recognized the unique qualities of this humble but extraordinarily accomplished scientist.

Footnotes

1. Although we always called Agustin, "Gus," as did his other collaborators. However, Gus once remarked with characteristic deadpan expression paired with smiling eyes that his friends of youth never called him Gus "because his name was Agustin." But, he never complained and hence we shall call him Gus.

References Cited

1. PLATT JL, VERCELLOTTI GM, DALMASSO AP, MATAS AJ, BOLMAN RM, NAJARIAN JS, et al. Transplantation of discordant xenografts: a review of progress. Immunology Today. 1990; 11: 450-456.

2. DALMASSO AP, VERCELLOTTI GM, PLATT JL, BACH FH. Inhibition of complement-mediated endothelial cell cytotoxicity by decay accelerating factor: potential for prevention of xenograft hyperacute rejection. Transplantation. 1991; 52: 530-533.

3. DALMASSO AP, MUELLER-EBERHARD HJ. INTERACTION OF AUTOLOGOUS COMPLEMENT WITH RED CELLS IN THE ABSENCE OF ANTIBODY. Proc Soc Exp Biol Med. 1964; 117: 643-650.

4. DALMASSO AP. On the intersections of basic and applied research in xenotransplantation. Xenotransplantation. 2012; 19: 137-143.

5. MULLER-EBERHARD HJ, NILSSON UR, DALMASSO AP, POLLEY MJ, CALCOTT MA. A molecular concept of immune cytolysis. Arch Pathol. 1966; 82: 205-217.

6. CRIST E, TAUBER AI. Debating humoral immunity and epistemology: the rivalry of the immunochemists Jules Bordet and Paul Ehrlich. J Hist Biol. 1997; 30: 321-356.

7. PILLEMER L, BLUM L, LEPOW IH, ROSS OA, TODD EW, WARDLAW AC. The properdin system and immunity: demonstration and isolation of a new serum protein, properdin, and its role in immune phenomena. Science. 1954; 120: 279-285.

8. NELSON RA, JR. An alternative mechanism for the properdin system. J Exp Med. 1958; 108: 515-535.

9. POLLEY MJ, MÜLLER-EBERHARD HJ. Chemistry and mechanism of action of complement. Prog Hematol. 1966; 5: 2-25.

10. YACHNIN S, RUTHENBERG JM. THE INITIATION AND ENHANCEMENT OF HUMAN RED CELL LYSIS BY ACTIVATORS OF THE FIRST COMPONENT OF COMPLEMENT AND BY FIRST COMPONENT ESTERASE; STUDIES USING NORMAL RED CELLS AND RED CELLS FROM PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA. J Clin Invest. 1965; 44: 518-534.

11. PICKERING RJ, WOLFSON MR, GOOD RA, GEWURZ H. Passive hemolysis by serum and cobra venom factor: a new mechanism inducing membrane damage by complement. Proc Natl Acad Sci U S A. 1969; 62: 521-527.

12. GEWURZ H, SHIN HS, MERGENHAGEN SE. Interactions of the complement system with endotoxic lipopolysaccharide: Consumption of each of the six terminal complement components. Journal of Experimental Medicine. 1968; 128: 1049-1057.

13. MARCUS RL, SHIN HS, MAYER MM. An alternate complement pathway: C-3 cleaving activity, not due to C4,2a, on endotoxic lipopolysaccharide after treatment with guinea pig serum; relation to properdin. Proc Natl Acad Sci U S A. 1971; 68: 1351-1354.

14. LACHMANN PJ, NICOL P. Reaction mechanism of the alternative pathway of complement fixation. Lancet. 1973; 1: 465-467.

15. HAMAD I, HUNTER AC, SZEBENI J, MOGHIMI SM. Poly(ethylene glycol)s generate complement activation products in human serum through increased alternative pathway turnover and a MASP-2-dependent process. Mol Immunol. 2008; 46: 225-232.

16. SILVERSTEIN AM. Autoimmunity versus horror autotoxicus: the struggle for recognition. Nat Immunol. 2001; 2: 279-281.

17. MERI S, PANGBURN MK. Discrimination between activators and nonactivators of the alternative pathway of complement: regulation via a sialic acid/polyanion binding site on factor H. Proc Natl Acad Sci U S A. 1990; 87: 3982-3986.

18. DONIN N, JURIANZ K, ZIPOREN L, SCHULTZ S, KIRSCHFINK M, FISHELSON Z. Complement resistance of human carcinoma cells depends on membrane regulatory proteins, protein kinases and sialic acid. Clin Exp Immunol. 2003; 131: 254-263.

19. DALMASSO AP, VERCELLOTTI GM, FISCHEL RJ, BOLMAN RM, BACH FH, PLATT JL. Mechanism of complement activation in the hyperacute rejection of porcine organs transplanted into primate recipients. American Journal of Pathology. 1992; 140: 1157-1166.

20. VERCELLOTTI GM, PLATT JL, BACH FH, DALMASSO AP. Enhanced neutrophil adhesion to xenogeneic endothelium via C3bi. Journal of Immunology. 1991; 146: 730-734.

21. DALMASSO AP, HE T, BENSON BA. Human IgM xenoreactive antibodies can induce resistance of porcine endothelial cells to complement-mediated injury. Xenotransplantation. 1996; 3: 54-62.

22. DALMASSO AP, BENSON BA, JOHNSON JS, LANCTO C, ABRAHAMSEN MS. Resistance against the membrane attack complex of complement induced in porcine endothelial cells with a Gal alpha(1-3)Gal binding lectin: up-regulation of CD59 expression. Journal of Immunology. 2000; 164: 3764-3773.

23. PLATT JL, VERCELLOTTI GM, LINDMAN BJ, OEGEMA TR, JR., BACH FH, DALMASSO AP. Release of heparan sulfate from endothelial cells. Implications for pathogenesis of hyperacute rejection. J Exp Med. 1990; 171: 1363-1368.

24. COOPER MD, PETERSON RDA, GOOD RA. Delineation of the thymic and bursal lymphoid systems in the chicken. Nature. 1965; 205: 143-146.

25. COOPER MD, GABRIELSEN AE, GOOD RA. Role of the thymus and other central lymphoid tissues in immunological disease. Annual Review of Medicine. 1967; 18: 113-139.

26. MILLER JF. The discovery of thymus function and of thymus-derived lymphocytes. Immunological Reviews. 2002; 185: 7-14.

27. MILLER JF. Immunological function of the thymus. Lancet. 1961; 2: 748-749.

28. GOOD RA, DALMASSO AP, MARTINEZ C, ARCHER OK, PIERCE JC, PAPERMASTER BW. The role of the thymus in development of immunologic capacity in rabbits and mice. J Exp Med. 1962; 116: 733-796.

29. DALMASSO AP, MARTINEZ C, SJODIN K, GOOD RA. Studies on the role of the thymus in immunobiology; reconstitution of immunologic capacity in mice thymectomized at birth. J Exp Med. 1963; 118: 1089-1109.

Figure Legends

Legend for Picture of A.P. Dalmasso
Agustin ("Gus") Dalmasso (1933-2021), Honorary Member of the International Xenotransplantation Association

Figure 1. The complement System in the early 1960s.

When Agustin Dalmasso began investigation of the complement system in the early 1960s, most thought activation of complement and cytolysis that ensued required binding of antibodies to antigenic sites on a cell surface, as depicted in the figure. From Archives of Pathology 82: 205-217, 1966 (5), with permission.

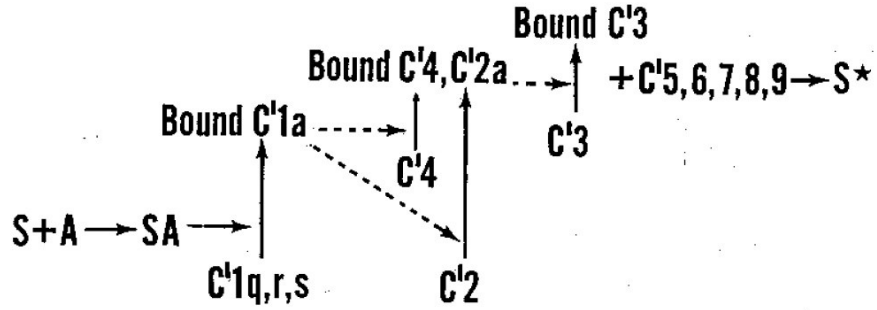

Fig 1.—Schematic representation of the complement reaction sequence. *S* denotes an area or site on the cell surface which is capable of binding antibody (*A*) and of reacting with complement. *S** represents a functional and structural lesion of the cell membrane.