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COMMENTARY

Systemic inflammation in xenograft recipients (SIXR) or undetected PCMV/PRV transmission?

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Transplantation of pig organs into non-human primates has often been associated with clinical symptoms described as systemic inflammation in xenograft recipients (SIXR).[1–4] Systemic inflammation precedes and promotes activation of coagulation after pig-to-nonhuman primate xenotransplantation, irrespective of immunosuppressive therapy.[3] Inflammation can be described as a complex biological response of an organism to harmful stimuli and chronic inflammation is observed in various diseases, for example, diabetes, infection, or atherosclerosis.[4] Inflammation is associated with the release of a wide range of cytokines and chemokines, recruiting immune cells to the site of inflammation and modulating the maturation of immune cells. Among these molecules are tumor necrosis factor (TNF) and interleukin 6 (IL-6), which are highly elevated in animals with SIXR.[5] Innate immune cells expressing tissue factor (TF) are activated after xenotransplantation and expression of the TF by activated endothelial cells is an initial mechanism in the development of thrombotic microangiopathy in the transplant and consumptive coagulopathy (CC) in the recipient.[3,6] Thrombotic microangiopathy and CC are characteristic features associated with xenotransplant failure.[7,8] TNF-alpha[9] and IL-6[10] are known to promote TF expression. SIXR is always associated with the activation of coagulation[1] and the interaction between inflammation and coagulation results in an amplification circuit,[11] promoting the production of both inflammatory mediators and coagulation factors. The influence of IL-6 on coagulation has been well studied in baboons.[10,12]

However, transmission of the porcine cytomegalovirus/porcine roseolovirus (PCMV/PRV) to non-human primate recipients of pig organs is also associated with CC.[13] The word transmission is used, because there is no evidence that PCMV/PRV infects human or non-human primate cells. Obviously, the virus replicates in the transplant and interacting with endothelial and lymphoid cells of the baboon, causing the changes in cytokine release and coagulation.[14] The name PCMV/PRV was chosen to indicate that this virus is not a cytomegalovirus, but a roseolovirus and that it is not closely related to the human cytomegalovirus (HCMV), also called human herpesvirus 5 (HHV-5), but that it is closely related to the roseoloviruses HHV-6 and HHV-7.[15] Most importantly, early weaning was shown to prevent PCMV/PRV transmission to the pigs, organs of PCMV/PRV-free animals obtained by early weaning did not induce CC.[16] An activation of the porcine TF in porcine aortic endothelial cells by a PCMV/PRV infection was observed in vitro.[13] Although no correlation between TF expression and PCMV/PRV infection was observed in vivo, decreased levels of PCMV/PRV activation were, however, associated with the prolongation of xenotransplant survival.[13,14] An expression of the TF by activated endothelial cells was also observed in reported cases of SIXR[3]; however, the expression of TF on immune cells (macrophages and peripheral blood mononuclear cells), another SIXR feature reported[3] was not yet studied after PCMV/PRV infection.[13] Interestingly, IL-6 was only elevated when immunosuppression was given,[1] indicating that PCMV/PRV could replicate to a titer able to induce IL-6 and coagulation dysfunction only under immunosuppression. When we analyzed baboons after the orthotopic transplantation of pig hearts with and without PCMV/PRV, we observed that the baboons, which presented clear pathological alterations, had very high levels of tissue-type plasminogen activator (tPA) – plasminogen activator inhibitor type 1 (PAI-1) (tPA-PAI-1) complexes.[14] This indicates a hypercoagulable state, defined as an increased tendency to develop blood clots (thrombosis). In an older report, it had been shown that IL-6 treatment induces a 20-fold increase of PAI-1 within 8 hours in a

baboon model.[10] Furthermore, the animals, which received a pig heart infected with PCMV/PRV, were characterized by a very high level of IL-6 in the serum.[14] In animals transplanted with a PCMV/PRV-free pig heart, neither high levels of IL-6 nor high levels of tPA-PAI-1 complexes were observed.

The strong similarity of the clinical symptoms described for animals with SIXR and for animals which have received a PCMV/PRV transmission raises the question as to whether the described SIXR represents an undetected PCMV/PRV transmission. The fact that PCMV/PRV induces similar features does not automatically mean that SIXR can be fully explained by a PCMV/PRV infection. Alternatively, a PCMV/PRV transmission may enhance SIXR. Unfortunately, the donor pigs in most of the investigations had not been tested with appropriate methods for PCMV/PRV detection, so they were believed to be PCMV/PRV-free, but were PCMV/PRV-positive in reality, as for example the donor pig of the heart for the patient in Baltimore.[17] After orthotopic transplantation of a heart from a PCMV/PRV-free donor pig, baboons do not show such features.[16] The transplantations of small (2 × 1 cm) pig artery patches into baboons[2,18] are of special interest. These patches certainly do not carry a high virus load, if any. However, they were able to induce C-reactive protein (CRP), a marker of inflammation,[19] as whole heart transplants also do, but this can be stopped by the application of an IL-6 blockade (tocilizumab). There are data showing that the small area of pig vascular endothelium stimulates a weaker coagulation/inflammation response compared with whole organs.[2] This is certainly due to the larger number of cells in the organs, but it may be also due to differences in the transmitted virus loads. Only future xenotransplantation experiments with truly PCMV/PRV-negative organs will give a final answer.

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CONFLICT OF INTEREST

The author has no conflict of interest.

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REFERENCES

1. Ezzelarab MB, Ekser B, Azimzadeh A, et al. Systemic inflammation in xenograft recipients precedes activation of coagulation. *Xenotransplantation*. 2015;22(1):32-47.
2. Iwase H, Ekser B, Zhou H, et al. Further evidence for sustained systemic inflammation in xenograft recipients (SIXR). *Xenotransplantation*. 2015;22(5):399-405.
3. Ezzelarab MB, Cooper DKC. Systemic inflammation in xenograft recipients (SIXR): a new paradigm in pig-to-primate xenotransplantation? *Int J Surg*. 2015;23(pt B):301-305.
4. Li J, Hara H, Wang Y, Esmon C, Cooper DKC, Iwase H. Evidence for the important role of inflammation in xenotransplantation. *J Inflamm (Lond)*. 2019;16:10
5. Zhang G, Iwase H, Li Q, et al. the role of interleukin-6 (IL-6) in the systemic inflammatory response in xenograft recipients and in pig kidney xenograft failure. *Front Immunol*. 2021;12:788949.
6. Lin CC, Ezzelarab M, Shapiro R, et al. Recipient tissue factor expression is associated with consumptive coagulopathy in pig-to-primate kidney xenotransplantation. *Am J Transplant*. 2010;10(7):1556-1568.
7. Bühler L, Basker M, Alwayn IP, et al. Coagulation and thrombotic disorders associated with pig organ and hematopoietic cell transplantation in nonhuman primates. *Transplantation*. 2000;70(9):1323-1331.
8. Cowan PJ, Robson SC, d'Apice AJ. Controlling coagulation dysregulation in xenotransplantation. *Curr Opin Organ Transplant*. 2011;16(2):214-221.
9. Kambas K, Markiewski MM, Pneumatikos IA, et al. C5a and TNF-alpha up-regulate the expression of tissue factor in intra-alveolar neutrophils of patients with the acute respiratory distress syndrome. *J Immunol*. 2008;180(11):7368-7375
10. Kruithof EK, Mestries JC, Gascon MP, Ythier A. The coagulation and fibrinolytic responses of baboons after in vivo thrombin generation effect of interleukin 6. *Thromb Haemost*. 1997;77(5):905-910.
11. Strukova S. Blood coagulation-dependent inflammation. Coagulation-dependent inflammation and inflammation-dependent thrombosis. *Front. Biosci. (Landmark Ed)*. 2006; 11(1), 59-80.
12. Mestries JC, Kruithof EK, Gascon MP, Herodin F, Agay D, Ythier A. In vivo modulation of coagulation and fibrinolysis by recombinant glycosylated human interleukin-6 in baboons. *Eur Cytokine Netw*. 1994;5(3):275-281.
13. Gollackner B, Mueller NJ, Houser S, et al. Porcine cytomegalovirus and coagulopathy in pig-to-primate xenotransplantation. *Transplantation* 2003;75(11), 1841-1847.</bib>
14. Denner J, Längin M, Reichart B, et al. Impact of porcine cytomegalovirus on long-term orthotopic cardiac xenotransplant survival. *Sci Rep*. 2020;10(1):17531.
15. Denner J, Bigley TM, Phan TL, Zimmermann C, Zhou X, Kaufer BB. Comparative analysis of roseoloviruses in humans, pigs, mice, and other species. *Viruses*. 2019;11(12):1108
16. Mueller NJ, Kuwaki K, Dor FJMF, et al. Reduction of consumptive coagulopathy using porcine cytomegalovirus-free cardiac porcine grafts in pig-to-primate xenotransplantation. *Transplantation*. 2004;78(10), 1449-1453.
17. Griffith BP, Goerlich CE, Singh AK, et al. Genetically modified porcine-to-human cardiac xenotransplantation. *N Engl J Med*. 2022;387(1):35-44.
18. Ezzelarab MB, Ekser B, Echeverri G, et al. Costimulation blockade in pig artery patch xenotransplantation – a simple model to monitor the adaptive immune response in nonhuman primates. *Xenotransplantation*. 2012;19(4):221-232.
19. Barrett TD, Hennan JK, Marks RM, Lucchesi BR. C-reactive-protein-associated increase in myocardial infarct size after ischemia/reperfusion. *J Pharmacol Exp Ther*. 2002;303(3):1007-1013.

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