*/6 2 9*

# SCREENING DONORS FOR XENOTRANSPLANTATION

## THE POTENTIAL FOR XENOZOONOSES[1]

MARIAN G. MICHAELS,[2,3] JOHN P. MCMICHAEL,[2] KATHLEEN BRASKY,[4] SEYMOUR KALTER,[5]
ROBERT L. PETERS,[6] THOMAS E. STARZL,[2] AND RICHARD L. SIMMONS[2]

*The University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; the Southwest Foundation for Biomedical Research, and the Virus Reference Laboratory, San Antonio, Texas; and Microbiological Associates, Inc., Rockville, Maryland*

**Xenotransplantation is a potential solution to the current donor shortage for solid organ transplantation. The transmission of infectious agents from donor organs or bone marrow to the recipient is a well-recognized phenomenon following allotransplantation. Thus the prospect of xenotransplantation raises the issue of xenozoonoses—i.e., the transmission of animal infections to the human host. Anticipating an increasing number of baboon to human transplants, 31 adult male baboons (*Papio cynocephalus*) from a single colony in the United States were screened for the presence of antibody to microbial agents (principally viral) that may pose a significant risk of infection. Antibody to simian cytomegalovirus, simian agent 8 and Epstein-Barr virus, was found in 97% of animals tested. Antibody to simian retroviruses and *Toxoplasma gondii* was found in 30% and 32% respectively. Discordant results were found when paired samples were examined by two primate laboratories. This was particularly noted when methodologies were based on cross-reaction with human viral antigens. These results highlight the need to develop specific antibody tests against the species used for xenotransplantation.**

Allotransplantation is an effective treatment for a variety of end-stage organ disorders. The number of people who can benefit from these procedures continues to grow as surgical techniques are refined and immunosuppressive regimens become more specific and efficient. A shortage of appropriate donors prompts the need to explore other donor sources. Xenotransplantation offers a potential solution to the current donor shortage for solid organ transplantation.

Infectious complications are a major cause of morbidity and mortality following allotransplantation (1, 2). Sources of infection include the recipient's normal flora or latently harbored organisms, environmental contamination, and organisms carried within the donor organ. Accordingly, a concern exists for inadvertent transmission of animal infections or xenozoonoses to the new human host during xenotransplantation.

The purpose of this screening study was to determine the seroprevalence of specific microbial agents in a baboon population that may pose a significant threat after xenotransplantation. The decision to screen for an organism was based on recognition that the analogous human organism causes donor-associated disease (1, 2), or that zoonotic transmission between primates and humans has been shown (3).

## METHODS

Thirty-one adult male baboons ranging in age between six and 16 years were screened serologically. All animals were raised at the Southwest Foundation for Biomedical Research in San Antonio, TX. The animals were housed in a six-acre corral containing approximately 450 baboons. Wild baboons have not been introduced into this colony for over ten years. Routine care included daily observation for signs of illness and tuberculin skin testing of animals and human care givers every six months.

*Toxoplasma gondii* serology was performed by Sabin-Feldman dye test at The Palo Alto Medical Foundation, Research Institute, Palo Alto, CA. Paired serum samples were sent for herpesvirus and retrovirus studies to two independent primate laboratories; Viral Reference Laboratory, Inc. (VRL),* San Antonio, TX and Microbiological Associates, Inc. (MA), Rockville, MD (Table 1). When available tests were specifically directed against the simian virus of interest (4, 5). If not available, laboratories relied on the crossreactivity of a test directed against the corresponding human virus. Positive and negative serum controls were performed with each test. In addition to serologic studies, plasma from 26 animals was inoculated by one laboratory (MA) into duplicate flasks of Raji cells to culture for simian AIDS retrovirus (SRV). Cultures were fed two times a week for two weeks and observed for typical syncytium formation. Suspect samples were incubated an additional seven days.

## RESULTS

Serologic tests on 30 of 31 animals were positive for cytomegalovirus, Epstein-Barr virus, and simian agent 8 (SA8). No single animal was seronegative for more than one herpesvirus. Discordant results (positive at one laboratory while negative at the other) were found for CMV (six animals) and SA8 (seven animals). Twenty-five discordant results were obtained for EBV serology.

Antibody to SIV was not detected by either laboratory in any animal. Antibody against simian T lymphotropic virus type 1 (STLV-1) was found in eight animals. SRV antibody was present in two animals; cultures were negative. All ani-

* Abbreviations: EBNA, Epstein-Barr nuclear antigen; HTLV-1, human T lymphotropic virus type 1; MA, Microbiological Associates; SA8, simian agent 8; SIV, simian immunodeficiency virus; SRV, simian retrovirus; STLV-1, simian T lymphotropic virus type 1; VRL, Viral Reference Laboratory.

TABLE 1. Results of viral studies performed on paired samples from 31 adult male baboons at two primate reference laboratories

| Virus | Microbiological Associates, Inc. (MA) | | Viral Reference Laboratory, Inc. (VRL) | | Total Positive from either laboratory (%) |
|---|---|---|---|---|---|
| | Method (human or primate antigen) | No. positive/ No. performed | Method (human or primate antigen) | No. positive/ No. performed | |
| Cytomegalovirus | ELISA[a]—Whittaker Bioproducts (human) | 19/25 | DIA—VRL (primate) | 30/31 | 97% |
| Epstein Barr virus | ELISA—Whittaker Bioproducts (EBNA) (human) | 1/26 | IFA—Gull Laboratory (VCA) (human) | 30/31 | 97% |
| Simian agent-8 | IFA—MA (primate) | 18/26 | DIA—VRL (primate) | 30/31 | 97% |
| Simian immunodeficiency virus | IFA—MA (primate) | 0/26 | DIA—VRL (primate) | 0/31 | 0 |
| Simian T lymphotropic virus | ELISA—Coulter Immunology Laboratory (human) | 5/26 | DIA—VRL (primate) | 5/31 | 24% |
| Simian AIDS retrovirus | IFA—MA (primate) | 1/26 | DIA—VRL (primate) | 1/31 | 6% |
| Hepatitis B virus | ELISA—Diagnostic Assay Service (human) | 1/26 | ELISA—Abbott Laboratory (human) | 0/31 | 3% |
| Hepatitis A virus | ELISA—Organon Teknika (human) | 2/26 | ELISA—Abbott Laboratory (human) | 3/31 | 10% |
| Foamy virus | IFA—MA (primate) | 16/23 | DIA—VRL (primate) | 30/31 | 97% |
| Lymphocytic choriomeningitis virus | IFA—MA (mouse) | 0/26 | DIA—VRL (mouse) | 0/31 | 0 |
| Simian hemorrhagic fever virus | Not performed | | DIA—VRL (primate) | 0/31 | 0 |
| Monkey pox virus | Not performed | | DIA—VRL (primate) | 0/31 | 0 |
| Measles | ELISA—Sigma Chemical Co. (human) | 12/26 | DIA—VRL (human) | 23/31 | 74% |
| Marburg virus | Not performed | | DIA—Center for Disease Control | 0/31 | 19% |

[a] ELISA: enzyme-linked immunosorbent assay; DIA: dot-immunobinding assay; EBNA, Epstein Barr virus nuclear antigen; VCA, Epstein Barr virus viral capsid antigen; IFA, indirect immunofluorescence assay.

mals except one were positive for foamy virus. Five discordant results were found for STLV.

Antibody to *T gondii* was found in ten animals. Antibody to hepatitis B surface antigen was found in a single animal and hepatitis A virus IgG was present in three baboons. No animal was positive for lymphocytic choriomeningitis virus, simian hemorrhagic fever virus, Marburg virus or monkey pox virus.

### DISCUSSION

The decision to screen for specific microbial organisms was based on (1) the knowledge that certain organisms are associated with donor transmitted infections after allotransplantation, (2) the concern that some endemic primate viruses are potentially fatal to humans, and (3) the availability of techniques to differentiate species specific viruses in order to document transmission of infection from primates to humans.

Members of the herpesvirus family such as CMV and EBV are well recognized as donor transmitted infections after allotransplantation (*1, 6, 7*). Antibody to these viruses were ubiquitous in our population of adult male baboons. A smaller study of ten wild baboons showed similar results for CMV but not positive antibody against EBV (*8*). In the current study, EBV results were highly discordant between laboratories both of which employed studies relying on crossreactivity of the baboon EBV-like organism, *herpesvirus papio* with human antibody. MA utilized a commercial ELISA directed against human EBV nuclear antigen (Whittaker Bioproducts), while VRL employed IFA directed against human EBV viral capsid antigen (Gull Laboratories). The discordant results may be explained by data from a previous study suggesting that baboon VCA has more crossreactivity with the human VCA than the EBNA (*9*). Also the recent sequencing of *herpesvirus papio* EBNA-2 shows significant divergence from human EBV EBNA-2 protein (*10*).

SA8 was found in 97% of animals. This virus is analogous to human herpes simplex virus causing self-limited, recurrent oral and genital lesions in primates. Acquisition is similar to that of herpes simplex in humans; increasing prevalence is found in primates with increasing age, particularly after sexual maturation (*11*). Serologic studies have been developed that distinguish SA8 from herpes simplex and *H. simiae* (B virus) (*12, 13*). While herpes simplex virus is not

considered a typical donor-associated pathogen, finding SA8 in a recipient would demonstrate transmission across species lines. Likewise, SA8 is similar to B virus, which is endemic in *Macaca* spp. and causes fatal disease in humans (*3*). SA8 has not been demonstrated to be transmitted from a baboon to a human. A recent report of positive B virus in baboons used a nonspecific assay directed against human herpes simplex that cannot distinguish the various viral species (*14*). The positive results were therefore likely due to crossreactivity with SA8 rather than with B virus.

Retroviruses can also be transmitted by organ transplantation (*15*). STLV-1, analogous to human T lymphotropic virus type 1 (HTLV-1) is found in as many as 40% of baboon populations studied (*16*). Because HTLV-1 has been associated with some forms of human T cell leukemias and an increased prevalence of lymphomas in macaque species (*17*) the presence of HTLV or STLV in potential xenograft donors is of importance. SRV has likewise been found in baboon populations in association with lymphomas (*18*). While simian immunodeficiency virus (SIV) analogous to human immunodeficiency virus type 2, is rare in baboon populations its detection would arouse great concern. While not included in this study, endogenous type C retroviruses are found in all baboons (*18*) and they or their genomic material represent an unknown infectious potential.

Foamy virus is a spumavirus-type retrovirus which has not been clearly identified as a cause of disease (*19*). It is uncommon in humans but highly prevalent in nonhuman primate species. Its presence in a recipient could be used to document transmission of a virus between species from xenotransplantation.

Hepatitis B antibody was found in one animal. Anti-Hbsag has been noted in several other serologic studies of wild and captive baboons (*20, 21*). While it is widely believed that baboons and many other nonhuman primates are not susceptible to infection with hepatitis B virus (*22*) these serologic findings should be verified with current molecular techniques.

Evidence of infection with *T gondii* was found in 32% of the baboon population studied. Antibody was detected in 16 of 100 wild baboons studied in South Africa (*23*). This parasite has been transmitted after solid organ allotransplantation (*1*). While heart graft recipients are at highest risk, liver and renal transplant recipients can also be infected from a seropositive donor. The risk of transmission of *T gondii* from an animal organ would be expected to be the similar to the risk after allotransplantation.

Serologic criteria for exclusion of an animal from a donor pool was used in our early xenotransplant experiments (*24*) to help limit the transmission of potential pathogens to the human transplant recipients. Animals with evidence of antibody against retroviruses other than foamy virus were excluded. *Toxoplasma*-positive animals were also excluded from the donor pool to avoid the additional need of prophylaxis with toxic chemotherapeutic agents. Foamy viruses have not been demonstrated to be pathogenic and serologic evidence of antibody to foamy virus was not used as an exclusionary factor for these early trials. Herpesviruses were ubiquitous in our population. CMV and EBV are generally considered to be species-specific (*25*). Although not a definite risk, there is potential for disease in the new host or reactivation within the xenograft itself. Antiviral agents do have activity against

primate herpesviruses and may prove useful in prophylactic or treatment strategies (*26*).

In summary, 30% of the adult animals tested had antibody against retroviruses and 32% against *Toxoplasma*. A total of 16 or 52% were disqualified as potential donors based on serological screening. Reliance on crossreactivity with human antibody tests may have led to variable results between laboratories. It will be important to develop sensitive, easy-to-perform assays directed against the organisms of a species chosen as a xenotransplant donor. Consideration has been given to raising primates in specific pathogen–free environments (*3*)—however, this would be logistically difficult and necessitate five to ten years because of the primate maturation period. Furthermore, neither our screening methods nor the development of specific pathogen–free animals would address the possibility of transmitting an as-yet-unrecognized primate organism to a naive human host (*3*). Follow-up surveillance cultures along with serologic evaluation of the human recipients of xenografts will be necessary to more accurately define the risk of xenozoonoses.

## REFERENCES

1. Ho M, Dummer JS. Risk factors and approaches to infections in transplant recipients. In: Mandell GC, Douglas GR Jr, Bennet JE, eds. Principles and practice of infectious diseases, 2nd ed. New York: Churchill Livingstone, 1990.
2. Green M, Michaels MG. Infectious complications of solid-organ transplantation in children. Adv Pediatr Infect Dis 1992; 7: 181.
3. Michaels MG, Simmons RL. Zoonoses and xenotransplantation: strategies for prevention. Transplantation (in press).
4. Kalter SS, Heberling RL. Viral battery testing in nonhuman primate colony management. Lab Anim Sci 1990; 40: 21.
5. Kalter SS. Overview of simian viruses and recognized virus diseases and laboratory support for the diagnosis of viral infection. In: Benirschke K, ed. Primates: The Road to self-sustaining populations. New York: Springer, 1986; 681.
6. Peterson PK, Balfour HH, Marker SC, Fryd DS, Howard RJ, Simmons RL. Cytomegalovirus in renal allograft recipients. Medicine 1980; 59: 283.
7. Cen H, Breinig MC, Atchinson RW, Ho M, McKnight JLC. Epstein-Barr virus transmission via the donor organs in solid organ transplantation: polymerase chain reaction and restriction fragment length polymorphism analysis of IR2, IR3 and IR4. J Virol 1991; 65: 976.
8. Van der Riet FdSJ, Human PA, Cooper DKC, et al. Virological consideration of the use of primates in xenotransplantation. Transplant Proc 1987; 19: 4068.
9. Falk L, Deinhardt F, Nonoyama M, et al. Properties of a baboon lymphotropic herpesvirus related to epstein barr virus. J Cancer 1976; 18: 798.
10. Ling PD, Ryon JL, Hayward SD. EBNA-2 of herpesvirus papio diverges significantly from the type A and type B EBNA-2 proteins of Epstein-Barr virus but retains an efficient transactivation domain with a conserved hydrophobic motif. J Virol 1993; 67: 2990.
11. Letvin JL, Hilliard JK, Lipper SL, Butler TM, Goodwin WJ. A naturally occurring epizootic of Simian agent 8 in the baboon. Lab Anim Sci 1988; 38: 394.
12. Katz D, Hilliard JK, Eberle R, Lipper SL. ELISA for detection of group common and virus-specific antibodies in human and simian sera induced by herpes simplex and related simian viruses. J Virol Methods 1986; 14: 99.
13. Heberling RL, Kalter SS. A rapid dot-immunobinding assay on nitrocellulose for viral antibodies. J Clin Microbiol 1986; 23: 109.

14. Chiche L, Adam R, Caillat-Zucman S, Castaing D, Bach J, Bismuth H. Xenotransplantation: baboons as potential liver donors? Transplantation 1993; 6: 1418.

15. Simmonds RJ, Holmberg SD, Hurwitz RL, et al. Transmission of human immunodeficiency virus type I from a seronegative organ and tissue donor. N Engl J Med 1992; 326: 726.

16. Mone' J, Whitehead E, Leland M, Hubbard G, Allan J. Simian T-cell leukemia virus type I infection in captive baboons. Aids Res Hum Retrovirus 1992; 8: 1667.

17. Homma T, Kanki PJ, King NW, et al. Lymphoma in macaques: association with virus of HTLV family. Science 1984; 225: 716.

18. Kalter SS, Heberling RL. Primate viral diseases in perspective. J Med Primatol 1990; 19: 519.

19. Weiss RA. Foamy retroviruses: a virus in search of a disease. Nature 1988; 333: 497.

20. Eichberg JW, Kalter SS. Hepatitis A and B: serologic survey of human and nonhuman primate sera. Lab Anim Sci 1980; 30: 541.

21. Peterson DA. Antibodies to hepatitis A and B in nonhuman primates. 1979; In: Abstracts of the annual meeting of the American Society for Microbiology. Los Angeles: American Society for Microbiology, 1979: 267.

22. Deinhardt F. Hepatitis in primates. Adv Virus Res 1976; 20: 113.

23. McConnell EE, Basson PA, Wolstenholme B, De Vos V, Malherbe HH. Toxoplasmosis in free ranging chacma baboons from the Kruger National Park. Trans R Soc Trop Med Hyg 1973; 67: 851.

24. Starzl TE, Fung J, Tzakis A, et al. Baboon-to-human liver transplantation. Lancet 1993; 341: 65.

25. Miller G. Epstein-Barr Virus: biology, pathogenesis, and medical aspects. In: Fields BN, Knipe DM, eds. Virology, 2nd ed. New York: Raven, 1990: 1921.

26. Boulter EA, Thornton B, Bauer DJ, Bye A. Successful treatment of experimental B virus (Herpesvirus simiae) infection with acyclovir. Br Med J 1980; 280: 681.