INFECTION AND IMMUNITY, May 2010, p. 2353–2354 Vol. 78, No. 5
0019-9567/10/$12.00 doi:10.1128/IAI.00072-10
Copyright © 2010, American Society for Microbiology. All Rights Reserved.

Letter to the Editor

An Epitope of *Bacillus anthracis* Protective Antigen That Is Cryptic in Rabbits May Be Immunodominant in Humans

In a recent article, Oscherwitz et al. endeavor to enhance the immunogenicity of a multiple antigenic peptide (MAP) vaccine that targets a loop-neutralizing determinant (LND) of *Bacillus anthracis* protective antigen (PA) (1). They showed in a previous study (2) that this MAP, consisting of four copies of amino acids 305 to 319 of PA (PA 305-319) extending from a lysine core, can elicit humoral immunity in rabbits that is specific and strongly neutralizing for the 2β2-2β3 loop in domain 2 of PA. However, LND-specific antibodies were not detected in rabbits immunized with whole PA, and it was concluded that PA 305-319 may be a cryptic epitope. T cell assays in PA 305-319-immunized mice showed that this peptide appeared to lack activity as a T helper cell epitope.

We have good evidence that humans exposed to PA, either following cutaneous anthrax infection or through vaccination, generate long-term, T cell memory to both whole PA and PA 305-319. In a study including samples from naturally exposed patients recovered from cutaneous anthrax, from vaccine-hyperimmunized AVP (anthrax vaccine precipitated) donors, and from recombinant PA-vaccinated donors, positive T cell gamma interferon (IFN-γ) enzyme-linked immunospot (ELISPOT) assay responses were detected across all groups on restimulation of peripheral blood mononuclear cells (PBMC) with both whole PA and PA 305-319 (Table 1; unpublished data). This shows that humans exposed to *B. anthracis* and whole PA recognize and generate memory to PA 305-319, suggesting that it is immunodominant rather than cryptic. Donors expressed a range of HLA haplotypes, with a majority being HLA-DR4 positive. While many important discoveries on the immunology of infection are made in experimental models, the human T cell response to PA in this respect differs from that of both rabbits and mice.

In this light, the epitope used by Oscherwitz and colleagues may indeed be more relevant to eliciting appropriate human immunity than previously envisaged.

REFERENCES

1. **Oscherwitz, J., F. Yu, and K. B. Cease.** 2009. A heterologous helper T-cell epitope enhances the immunogenicity of a multiple-antigenic-peptide vaccine targeting the cryptic loop-neutralizing determinant of *Bacillus anthracis* protective antigen. Infect. Immun. **77:**5509–5518.
2. **Oscherwitz, J., F. Yu, J. L. Jacobs, T. H. Liu, P. R. Johnson, and K. B. Cease.** 2009. Synthetic peptide vaccine targeting a cryptic neutralizing epitope in domain 2 of *Bacillus anthracis* protective antigen. Infect. Immun. **77:**3380–3388.

Rebecca J. Ingram
School of Medicine, Dentistry and Biomedical Sciences
Queen's University Belfast
Belfast, United Kingdom

Karen K. Chu
Department of Infectious Diseases and Immunity
Imperial College London
London, United Kingdom

Gökhan Metan
Department of Infectious Disease
Erciyes University Hospital
Kayseri, Turkey

Bernard Maillere
CEA
iBiTecS
Service d'Ingénierie Moléculaire des Protéines (SIMOPRO)
Gif Sur Yvette, France

Mehmet Doganay
Department of Infectious Disease
Erciyes University Hospital
Kayseri, Turkey

Yusuf Ozkul
Department of Medical Genetics
Erciyes University Hospital
Kayseri, Turkey

Hugh Dyson
E. Diane Williamson
Defence Science Technology Laboratory
Porton Down
Salisbury, United Kingdom

Les Baillie
Welsh School of Pharmacy
Cardiff University
Cardiff, United Kingdom

Louise U. Kim
Stephanie Ascough
Shiranee Sriskandan
Department of Infectious Diseases and Immunity
Imperial College London
London, United Kingdom

Daniel M. Altmann*
Department of Infectious Diseases and Immunity
Imperial College London
Hammersmith Hospital
Du Cane Road
London W12 0NN, United Kingdom

*Phone: 44 208 383 8423
E-mail: d.altmann@imperial.ac.uk

Authors' Reply

Ingram and colleagues present interesting evidence that the 305-319 peptide from PA is recognized by human T cells from PA vaccinees and anthrax survivors as evidenced by ELISPOT assays. We are not surprised that this segment would be seen by T cells in some human subjects and believe that more extensive examination of the human T cell response to PA in general and with regard to the LND specifically would be of significant interest.

We utilized inbred mice to model the likely performance of our LND immunogens in outbred rabbits. Our data demonstrated that the LND peptide was devoid of helper T cell activity in multiple strains of inbred mice. This determination led us to evaluate the immunogenicity in rabbits of the LND peptide linked to a heterologous source of helper T cell stimulation, since it has long been established that T cell-depen-

Downloaded from https://journals.asm.org/journal/iai on 24 February 2022 by 193.140.39.39.

TABLE 1. T cell IFN-γ responses to PA 305-319 in PBMC of vaccinated and naturally exposed subjects

Donor[a]	HLA-DR type	Response to PA 305-319[b]
CA1	4, 14	+
CA2	4, 14	+
AVP1	11, 13	+
AVP2	7, 15	+
rPA1	4, 17	+
rPA2	4, 9	+
rPA3	4, 15	+

[a] CA, naturally exposed patient recovered from cutaneous anthrax; AVP, AVP vaccine-hyperimmunized donor; rPA, recombinant-PA-vaccinated donor.
[b] Evaluated by IFN-γ ELISPOT of peptide PA 305-319-restimulated PBMC cultures. +, positive T cell response defined as spot-forming cell number greater than 2 standard deviations from the no-peptide control triplicates.

dent antibody responses to haptenic peptide sequences require a covalently linked source of helper T cell stimulation (6). Indeed, our data for rabbits demonstrated that the immunogenicity of the LND peptide linked to a helper T cell epitope from tetanus toxoid was significantly higher than the immunogenicity of the peptide without a linked source of T cell help (4, 5). It is important to note, however, that our studies did not examine whether the 305-319 sequence is capable of stimulating T cells in rabbits or in humans. While the ELISPOT data indicate that the 304-319 sequence can be recognized by human T cells, we would be reluctant to conclude that the 304-319 sequence is immunodominant in humans without clear demonstration that the recognition of this peptide by T cells is comparatively superior to the recognition of most other peptide sequences processed from within PA. If future studies were to confirm that the 305-319 sequence is immunodominant in humans, this could have implications for the use of this peptide in anthrax vaccines, especially in regard to the induction of anamnestic immunity among LND vaccinees exposed to PA.

We believe it would be helpful to clarify the usage of the term "cryptic" in our papers. Our data show that the LND peptide sequence appears to be immunologically silent or cryptic in PA as a target for humoral immunity in rabbits; immunization of rabbits with PA does not stimulate antibody to the LND. Evidence also exists that the LND may be cryptic at the level of the B cell in humans immunized with PA. Cryptic B cell epitopes have been reported in many proteins, including in the circumsporozoite protein of *Plasmodium falciparum* (7), in HIV gp120 (1), and in the hemagglutinin protein of influenza virus (2, 8), among others. The explanation for the crypticity of the LND is not currently known, but may be related to complex protein structural features associated with the 2β2-2β3 loop in domain 2 of PA where this sequence is found (3).

REFERENCES

1. **Bou-Habib, D. C., G. Roderiquez, T. Oravecz, P. W. Berman, P. Lusso, and M. A. Norcross.** 1994. Cryptic nature of envelope V3 region epitopes protects primary monocytotropic human immunodeficiency virus type 1 from antibody neutralization. J. Virol. **68:**6006–6013.
2. **Ekiert, D. C., G. Bhabha, M. A. Elsliger, R. H. Friesen, M. Jongeneelen, M. Throsby, J. Goudsmit, and I. A. Wilson.** 2009. Antibody recognition of a highly conserved influenza virus epitope. Science **324:**246–251.
3. **Lacy, D. B., D. J. Wigelsworth, R. A. Melnyk, S. C. Harrison, and R. J. Collier.** 2004. Structure of heptameric protective antigen bound to an anthrax toxin receptor: a role for receptor in pH-dependent pore formation. Proc. Natl. Acad. Sci. U. S. A. **101:**13147–13151.
4. **Oscherwitz, J., F. Yu, and K. B. Cease.** 2009. A heterologous helper T-cell epitope enhances the immunogenicity of a multiple-antigenic-peptide vaccine targeting the cryptic loop-neutralizing determinant of Bacillus anthracis protective antigen. Infect. Immun. **77:**5509–5518.
5. **Oscherwitz, J., F. Yu, J. L. Jacobs, T. H. Liu, P. R. Johnson, and K. B. Cease.** 2009. Synthetic peptide vaccine targeting a cryptic neutralizing epitope in domain 2 of Bacillus anthracis protective antigen. Infect. Immun. **77:**3380–3388.
6. **Rajewsky, K., V. Schirrmacher, S. Nase, and N. K. Jerne.** 1969. The requirement of more than one antigenic determinant for immunogenicity. J. Exp. Med. **129:**1131–1143.
7. **Rathore, D., R. Nagarkatti, D. Jani, R. Chattopadhyay, P. de la Vega, S. Kumar, and T. F. McCutchan.** 2005. An immunologically cryptic epitope of Plasmodium falciparum circumsporozoite protein facilitates liver cell recognition and induces protective antibodies that block liver cell invasion. J. Biol. Chem. **280:**20524–20529.
8. **Sui, J., W. C. Hwang, S. Perez, G. Wei, D. Aird, L. M. Chen, E. Santelli, B. Stec, G. Cadwell, M. Ali, H. Wan, A. Murakami, A. Yammanuru, T. Han, N. J. Cox, L. A. Bankston, R. O. Donis, R. C. Liddington, and W. A. Marasco.** 2009. Structural and functional bases for broad-spectrum neutralization of avian and human influenza A viruses. Nat. Struct. Mol. Biol. **16:**265–273.

Jon Oscherwitz*
Kemp B. Cease
University of Michigan Medical School
Ann Arbor Veterans Affairs Medical Center
Ann Arbor, Michigan 48105

*Phone: (734) 845-5195
Fax: (734) 845-3246
Email: joscher@umich.edu

Editor: R. P. Morrison

Downloaded from https://journals.asm.org/journal/iai on 24 February 2022 by 193.140.39.39.