

Does C-2 kinin exist?

To the Editor:

The September issue of the Journal and the accompanying supplement focused on the substantial progress that has been made in understanding the mechanisms by which swelling occurs in C1 inhibitor deficiency and in its treatment. We wish to comment on the pathway for kinin formation that was provided by Bowen et al¹ and used as the cover illustration. It indicates that factor XII-dependent generation of bradykinin is now thought to be the major mediator of the swelling and that any role for C-2 kinin is questionable. In the supplement article by Agostoni et al,² 2 references are given as being among the first to call attention to bradykinin of C-2 kinin as mediators of the swelling,^{3,4} but their respective contributions were not distinguished. This is followed by a series of references providing further evidence in support of a role for bradykinin. The message in our article⁵ was that plasma of patients with C-1 inhibitor deficiency is unstable and readily liberates bradykinin on incubation, even in plastic, a condition that does not activate normal plasma. We also provided evidence that cleavage of C-2 does not yield a kinin even in the presence of plasmin. It has taken 17 years since our publication in 1987 to see doubt raised by others regarding the role of C-2 kinin in this disease. But one wonders why, in 2004, it is even mentioned as a possibility. The original observation was made in 1977 and indicates that activation of complement followed by cleavage of a C-2 fragment by plasmin leads to kinin formation,⁴ and that the process is factor XII-dependent.⁵ However, 27 years have passed and no peptide has been isolated, no assay developed, and neither they nor anyone else has ever reproduced the observation. The closest we have come is a report by Strang et al,⁶ who synthesized overlapping peptides from C-2 and found one that had kininlike activity, but C-2 is not cleaved by any known enzyme to release it. Our attempts to cleave C-2 in the presence of plasmin failed to release a kinin,³ and every article since has provided increasing evidence that bradykinin is the only kinin produced when such plasma is activated, including a subsequent article by one of the original authors,⁷ or when kinins are examined in *in vivo* studies of patients.⁸ In addition, a family with a mutant C-1 inhibitor that lacks function in the complement cascade but retains inhibitory activity on bradykinin-forming enzymes has no family members with swelling,⁹ whereas an animal model of C-1 inhibitor deficiency revealed that a bradykinin type 2 receptor antagonist inhibits the resultant vascular permeability.¹⁰

We concede that stopping attacks of swelling in patients using a specific bradykinin receptor antagonist or identifying hereditary angioedema in a patient who also has a congenital C-2 deficiency would be even more definitive. Nevertheless, the totality of the data we have and the absence of any progress in characterizing a kinin derived from C-2 should lead to the conclusion that the

original observation is more likely incorrect than simply questionable. Aspects of C-1 inhibitor deficiency that are not yet understood may relate to information regarding bradykinin formation or function that we do not yet understand, or even the presence of some as yet undiscovered vasoactive substance, but does not seem to be a reason to retain even the question of a kinin from C-2 unless someone provides new evidence for its existence.

Allen P. Kaplan, MD
Medical University of South Carolina
Department of Medicine
Division of Pulmonary/Allergy
96 Jonathan Lucas St
Charleston, SC 29425
Berhane Ghebrehiwet, DVM
State University of New York at Stony Brook
Stony Brook, NY

REFERENCES

1. Bowen T, Cicardi M, Farkas H, Bork K, Kreuz W, Zingale L, et al. Canadian 2003 International Consensus Algorithm for the diagnosis, therapy, and management of hereditary angioedema. *J Allergy Clin Immunol* 2004;114:629-37.
2. Agostoni A, Aygoren-Pursun E, Binkley KE, Blanch A, Bork K, Bouillet L, et al. Hereditary and acquired angioedema: problems and progress: proceedings of the third C-1 esterase inhibitor deficiency workshop and beyond. *J Allergy Clin Immunol* 2004;114(suppl):S51-S131.
3. Fields T, Ghebrehiwet B, Kaplan AP. Kinin formation in hereditary angioedema plasma: evidence against kinin derivation from C-2 and in support of "spontaneous" formation of bradykinin. *J Allergy Clin Immunol* 1983;72:54-60.
4. Donaldson VH, Rosen FS, Bing DH. Role of the second component of complement (C-2) and plasmin in kinin-release in hereditary angioneurotic edema (H.A.N.E.) plasma. *Trans Assoc Am Physicians* 1977;90:74-83.
5. Donaldson VH. Mechanisms of activation of C-1 esterase in hereditary angioneurotic edema plasma in-vitro: the role of Hageman factor, a clot-promoting agent. *J Exp Med* 1968;127:411-29.
6. Strang CJ, Cholin S, Spragg J, Davis AE III, Schneeberger CE, Donaldson VH, et al. Angioedema induced by a peptide derived from complement component C-2. *J Exp Med* 1998;168:1685-98.
7. Shoemaker LR, Schurman SJ, Donaldson VH, Davis AH III. Hereditary angioneurotic oedema: characterization of plasma kinin and vascular permeability-enhancing activities. *Clin Exp Immunol* 1994;95:22-8.
8. Nussberger J, Cugno M, Cicardi M, Agostoni A. Local bradykinin generation in hereditary angioedema. *J Allergy Clin Immunol* 1999;104:1321-2.
9. Zahedi R, Bissler JJ, Davis AE III, Andreadis C, Wisniewski JJ. Unique C-1 inhibitor dysfunction in a kindred without angioedema, II: identification of a Ala Val substitution and functional analysis of the recombinant mutant protein. *J Clin Invest* 1995;95:1299-305.
10. Han ED, MacFarlane RC, Mulligan AN, Scafidi J, Davis AE III. Increased vascular permeability in C-1 inhibitor deficiency mice mediated by the bradykinin type 2 receptor. *J Clin Invest* 2002;109:1057-63.

doi:10.1016/j.jaci.2004.12.1132

Drug presentation to T cells

To the Editor:

We found the results presented by Nassif et al¹ regarding activation of sulfamethoxazole specific CD8⁺ T cells in toxic epidermal necrolysis very interesting. However, we think that their comments regarding the role of drug

metabolism in T-cell activation are not fully supported by the data presented.

The data presented by Nassif et al¹ show that blister fluid cells from 2 patients were stimulated by both sulfamethoxazole and the protein-reactive metabolite nitroso sulfamethoxazole. Importantly, T-cell stimulation was observed with high concentrations of the parent drug (100 µg/mL), which are obtained therapeutically only in patients with HIV infection receiving sulfamethoxazole for treatment of *Pneumocystis pneumonia*, whereas the concentration of nitroso sulfamethoxazole was 20-fold lower (5 µg/mL). Because nitroso sulfamethoxazole at a concentration of 1 µg/mL did not stimulate a response, the authors concluded that only the parent drug has a primary role in sulfamethoxazole immunogenicity. The validity of this conclusion rests on the observation that approximately 2% of an ingested dose of sulfamethoxazole is excreted as oxidative metabolites in urine.

However, this is not an accurate method by which to estimate the degree of the formation of reactive metabolites *in vivo* and thereby the cellular exposure. Indeed, it is likely that this may greatly misjudge cellular exposure to nitroso sulfamethoxazole because the analysis does not account for the possibility of drug accumulation, covalent binding to tissues, or organ-specific metabolic activation. In this respect, both skin and immune cells metabolize sulfamethoxazole. Furthermore, nitroso sulfamethoxazole undergoes extensive and rapid reduction to sulfamethoxazole; thus, analysis of urinary excretion of unstable metabolites is not a reliable index of tissue exposure.

In addition, when performing *in vitro* cell culture experiments, one must consider the stability of nitroso sulfamethoxazole, and thus the extent of exposure of cells to the drug metabolite as a function of the area under the curve. Previously, we have shown that only trace amounts of nitroso sulfamethoxazole are detectable after 5 minutes in cell culture medium.² Thus, taking account of the time necessary to prepare necessary stock solutions, the concentration of nitroso sulfamethoxazole that cells are exposed to is, at a best estimation, 10-fold to 100-fold lower than that described in the article by Nassif et al.¹

Finally, to appraise fully the role of metabolism in the stimulation of sulfamethoxazole-specific T cells, one must consider the following: first, T-cell responses from sulfamethoxazole-hypersensitive patients have, without failure, been detected with both the parent drug and the protein-reactive nitroso metabolite^{3,4}; second, a primary response using T cells from drug-naïve volunteers was observed with nitroso sulfamethoxazole in 9 of 10 individuals, whereas only 3 reacted to the parent drug⁵; and finally, in animal models of sulfamethoxazole immunogenicity, nitroso sulfamethoxazole but not the parent drug was found to be antigenic.²

Thus, although it is beyond doubt that *in vitro*, non-covalently bound sulfamethoxazole stimulates T cells from hypersensitive individuals, the role of sulfamethoxazole metabolite-specific T cells in the clinical manifestations of toxic epidermal necrolysis and drug hypersensitivity in general should not be discounted so readily.

Dean John Naisbitt, PhD
Munir Pirmohamed, FRCP
Brian Kevin Park, PhD
Department of Pharmacology
University of Liverpool
Sherrington Building
Ashton Street
Liverpool, Merseyside
L69 3GE, England

REFERENCES

1. Nassif A, Bensussan A, Boumsell L, Deniaud A, Moslehi H, Wolkenstein P, et al. Toxic epidermal necrolysis: effector cells are drug-specific cytotoxic T-cells. *J Allergy Clin Immunol* 2004;114:1209-15.
2. Naisbitt DJ, Farrell J, Gordon SF, Maggs JL, Burkhardt C, Pichler WJ, et al. Covalent binding of the nitroso metabolite of sulfamethoxazole leads to toxicity and major histocompatibility complex-restricted antigen presentation. *Mol Pharmacol* 2002;62:628-37.
3. Schnyder B, Burkhardt C, Schnyder-Frutig K, von Greysen S, Naisbitt DJ, Pirmohamed M, et al. Recognition of sulfamethoxazole and its reactive metabolites by drug-specific CD4+ T cells from allergic individuals. *J Immunol* 2000;164:6647-54.
4. Farrell J, Naisbitt DJ, Drummond NS, Depta JP, Vilar FJ, Pirmohamed M, et al. Characterization of sulfamethoxazole and sulfamethoxazole metabolite-specific T-cell responses in animals and humans. *J Pharmacol Exp Ther* 2003;306:229-37.
5. Engler OB, Strasser I, Naisbitt DJ, Cerny A, Pichler WJ. A chemically inert drug can stimulate T cells *in vitro* by their T cell receptor in non-sensitized individuals. *Toxicology* 2004;197:47-56.

doi:10.1016/j.jaci.2005.01.011

Reply

To the Editor:

Naisbitt et al¹ question the concentration of 100 µg/mL sulfamethoxazole (SMX) in experiments that demonstrated SMX specific cytotoxicity of CD8 T cells from the skin lesions of patients with toxic epidermal necrolysis.² This concentration is indeed at the upper limit of therapeutic serum levels in humans. Prior *in vitro* studies, including one coauthored by Naisbitt et al, tested lymphocytes with SMX concentrations ranging from 20 to 250 µg/mL.³ Lacking cells to use multiple doses, we chose 100 µg/mL as a concentration capable of optimally stimulating specific T cells³ while still in therapeutic range.

Second, they challenge our interpretation of results obtained with reactive metabolites of SMX. Cytotoxic T cells were activated by 5 µg/mL and 20 µg/mL of the nitroso metabolite (SMX-NO) but not by 1 µg/mL (ie, 4 µmol/L), a concentration that we considered more physiologic. The epidermis contains no vessels, and because of high chemical reactivity, it is unlikely that free and still reactive SMX-NO can reach epidermal cells from the blood across the basement membranes of dermal capillaries and the dermo-epidermal junction. Human keratinocytes incubated with 1000 µmol/L SMX (about 250 µg/mL) produced 0.001 µmol/L SMX hydroxylamine, a precursor of SMX-NO.⁴ Therefore, using *in vitro* 4 µmol/L SMX-NO was probably closer to a physiologic situation than higher doses. But our negative results could be explained also by technical pitfalls, including