

IDTs observed with 2 PS, never used before, were considered as possibly false-positive results.

We decided to readminister cisplatin: 1/100 of the required dosage, then 1/10, and on the third day, the full dose associated with betamethasone and antiemetic drugs. This readministration was well tolerated, as were the following 5 courses of chemotherapy.

Whereas IDTs with PS have a good negative predictive value,^{1,4} especially in immediate reactions to PS, in delayed skin reactions occurring with chemotherapies, IDTs with PS have to be considered with caution because they can induce false-positive results.

Because the chemotherapy had to be continued, IDTs were performed a few days after the disappearance of the maculopapular rash. This shorter than usual delay,³ required for investigating adverse drug reactions in oncology,⁵ could be responsible for this false-positive IDT.

As previously published,⁵ in managing a rash occurring during chemotherapy, associated treatments have to be taken into account and tested.

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Kinin formation in C1 inhibitor deficiency

To the Editor:

Bossi et al¹ have reported novel and potentially important observations regarding the pathogenesis of hereditary angioedema (HAE), including a possible role for the bradykinin B-1 receptor, as well as gC1qR, an endothelial receptor that binds high-molecular-weight kininogen (HK) and Factor XII.² Antiserum directed to the HK/Factor XII binding site of gC1qR and inhibitors of the B-1 receptor diminished vascular permeability caused by endothelial cell exposure (*in vitro* and *in vivo*) to HAE plasma of symptomatic patients. Because the B-1 receptor is induced in

endothelial cells, it is assumed that the HAE plasma contains 1 or more cytokines (some evidence for IL-1 is presented) that up-regulate the B-1 receptor to account for the reported time lag in enhancement of permeability. The contribution of gC1qR to the increase in vascular permeability is more difficult to understand. There is no known interaction of gC1qR with the B-2 or B-1 bradykinin receptors, but a control is missing to check whether anti-gC1qR affects vascular permeability induced by bradykinin. Assuming that these receptors do not cross-activate or inhibit, the anti-gC1qR must be inhibiting the interaction of HK or Factor XII with the receptor. Because the HAE attack plasma has markedly increased kallikrein levels,³ it could activate any HK or Factor XII bound to the gC1qR along the surface of endothelial cells. Then bradykinin would be liberated, and some might be degraded to the B-1 receptor agonist des-arg⁹ bradykinin by carboxypeptidase N. Thus we cannot be sure whether the permeability stimulus is contained in the HAE plasma or is generated during the course of the experiments. Direct measurement of these kinin agonists in the HAE plasma would help settle the issue.

The idea that inhibiting gC1qR might ameliorate symptoms or prevent angioedema attacks in patients with HAE is enticing because gC1qR is a key component of the 2 bimolecular complexes⁴ that mediate binding of HK and Factor XII to endothelial cells so that activation can be localized to a particular site.⁵ The authors demonstrate that a B-1 receptor agonist alone did not cause much increase in vascular permeability compared with bradykinin but that inhibition of both receptors was a striking improvement over blockade of the B-2 receptor alone. Now that we have agents that target the plasma bradykinin-forming cascade, namely ecallantide, a kallikrein inhibitor, and icatibant, a B-2 receptor antagonist,^{6,7} for treatment of acute attacks of swelling, additional approaches to improved treatment and prophylaxis would be of considerable interest. Regardless, future studies need to pinpoint the concentration of the 2 kinin moieties directly in the HAE plasma and then continue to define what the contribution of B-1 receptor stimulation might be because the positive result reported with anti-gC1qR casts doubt on the source of the kinins being measured. The result notwithstanding, a trial of anti-gC1qR in patients as an alternate approach to prophylaxis (ie, intravenous C1 INH) would be of considerable interest.

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Reply

To the Editor:

We would like to thank Drs Kaplan and Joseph¹ for recognizing the novelty of our work and the importance of our observations in the understanding of the mechanisms responsible for the onset of acute attacks in patients with hereditary angioedema (HAE). When we started testing the plasma samples collected during the attack phase, we were surprised to see that they failed to induce prompt vascular leakage as expected from the data published by Nussberger et al² showing increased levels of bradykinin in the attack-phase plasma. This finding led us to believe that the permeabilizing factor or factors are not present in the HAE plasma but are generated in the course of the experiment. This does not mean that bradykinin is not released in measurable amounts in the plasma during the attack phase, but it becomes quickly biologically inactive. For these reasons, we think that measuring the concentration of the kinins in the HAE plasma is not useful to provide an indication of a direct involvement in the vascular leakage.

Kaplan and Joseph¹ also raised the issue of the role played by gC1qR in increasing the vascular permeability in our model system. Having ruled out the contribution of preformed kinins in the HAE attack plasma and based on the complete inhibition of vascular permeability obtained with the mAb specific to the high-molecular-weight kininogen site on gC1qR, we reckon that the interaction of this receptor with high-molecular-weight kininogen/Factor XII is the primary step in the release of bradykinin and the degraded form des-arg⁹ bradykinin. Because vascular leakage is not induced by the remission-phase plasma, we hypothesize that proinflammatory molecules, such as IL-1, present in the attack-phase plasma might not only be responsible for the membrane expression of the B-1 receptor but also cause an increased expression of gC1qR that in turn leads to the release of an amount of kinins sufficient to promote vascular permeability.

To date, we have no direct evidence that there is an interaction between gC1qR and the B-1 or B-2 receptors, although our more recent data definitely exclude that the antibody to gC1qR inhibits bradykinin-induced vascular permeability. There is no doubt that a trial is needed to ascertain whether the patients would benefit from a combined treatment with B-2 and B-1 receptor antagonists. Another approach would be to treat patients with a blocking antibody to gC1qR, and we agree with Kaplan and Joseph¹ that a clinical trial is required to evaluate its *in vivo* efficacy.

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Ovalbumin content of influenza vaccines

To the Editor:

Some 2009-2010 influenza vaccine package inserts indicate that each dose may contain up to 1 µg ovalbumin; others do not provide information on ovalbumin content. The ovalbumin content of influenza vaccines is important if these vaccines are administered to patients with egg allergy. In a previous publication¹ we (M.A.R. and J.T.L.) proposed a protocol for administration of influenza vaccine to patients with egg allergy when the ovalbumin content of the vaccine is unknown.

Previous study of seasonal influenza vaccines showed significant variability in ovalbumin content among vaccines of different manufacturers.^{2,3} Recently, Waibel and Gomez⁴ used a commercial ovalbumin ELISA kit to assay ovalbumin concentrations in a sampling of twelve 2009-2010 seasonal and H1N1 influenza vaccines. We extend the results of Waibel and Gomez⁴ by reporting the ovalbumin content of additional 2009 seasonal and 2009 H1N1 influenza vaccines. Further, we report our initial results of lot-to-lot variability.

Multiple lots from different vendors of seasonal and H1N1 flu vaccines were received from several clinics across the United States and Japan. Vaccines were collected over a 2-month period. These were tested at 2 or more dilutions.

Purified rabbit polyclonal antiovalbumin antibodies were purchased from Novus Biologicals (Littleton, Colo). A horseradish peroxidase-conjugated rabbit polyclonal antibody to ovalbumin was purchased from Abcam (Cambridge, Mass) and used as a detection antibody. The capture antibody (Novus NB600-922) was coated to a 96-well microtiter plate (Immulon 4HBX, Fisher Scientific, Rockford, Ill) at 3 µg/mL in carbonate-bicarbonate buffer (Pierce #28382, Fisher Scientific) overnight at room temperature. After washing with wash buffer (0.5% Tween-20 in PBS, pH 7.4), wells were blocked with 1% normal rabbit serum in PBS for 1 hour at room temperature. Standards (chicken ovalbumin, grade VI; Sigma, St Louis, Mo), controls, and samples were added to the microtiter plate in duplicate and incubated for 2 hours at room temperature. After washing, detection antibody labeled with horseradish peroxidase (Abcam ab20415, diluted 1:50,000) was added and incubated at room temperature for 1 hour. The plate was washed, and the tetramethyl benzidine substrate was added for 5 to 10 minutes. The reaction was stopped with 2 N H₂SO₄, and absorbance at 450 nm was measured with a microplate reader (SpectraMax Plus; Molecular Devices, Sunnyvale, Calif). The lowest point of the standard curve was 0.19 ng/mL. The recovery of ovalbumin, which was spiked into the vaccine specimens, was 95% ± 6% (mean ± SEM; n = 16).

Fifty-eight samples (varying manufacturers and lots) of 2009 influenza vaccines were assayed for ovalbumin concentration: 35 seasonal influenza vaccines and 23 H1N1 vaccines. Our study included a larger number of samples than the report by Waibel and Gomez,⁴ which permitted an initial assessment of lot-to-lot variability of 2 vaccines. Our study also differs somewhat from the