

LETTERS TO THE EDITOR

To the Editor:

We want to comment on the article written by Wustrow, T. and Zenner, H., entitled "Natural Killer Cell Activity in Patients with Carcinoma of the Larynx and Hypopharynx."

We are particularly interested in the trypan blue technique used by the authors to measure NK cell activity. We have tried to develop a new method based on trypan blue uptake by dead tumor cells which substitutes ⁵¹Cr-release assay. The trypan blue method has a major disadvantage: it leads to an inaccurate count of cells because of dead cell liability. This phenomenon is a result of swelling of dead tumor cells which obscures the membrane boundary and faints the trypan blue color. We believe that this may be the reason why there are no differences in NK cell activity between controls and test groups in the above mentioned article.

Sincerely,
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Dear Editor:

We are grateful for the comments made by Drs. Ghoneum, Gill, and Perry regarding the trypan blue technique.

Natural killer cell activity is determined as the K562 cell killing by a small lymphocyte subpopulation. Thus the cell lysis has to be measured.

The Cr-release assay has been used in addition to the trypan blue exclusion test for many years in our laboratories.¹ Very well aware of the different methods, we have analyzed the first patient groups of our NK cell study in parallel with both techniques: No differences in NK cell activity could be detected. There is, however, a distinct spontaneous Cr-release and damage of cells during the incubation period leading to a *variable* background which always has to be corrected. Furthermore, ⁵¹Cr shows a measurable decline in activity due to radioactive degradation; thus, there is a considerable day to day and intraindividual variation which may exceed the measurable accuracy in the Cr assay.²

On the other hand, there is hardly any background with trypan blue, which is only incorporated into dead cells if the cell membrane barriers are not any more intact. In addition, the trypan blue uptake reflects the true cell viability, since cells, which are lethally hit, do take up trypan blue early, but do release Cr only until cell rupture is achieved. The security of the trypan blue technique is increased as

well by the possible microscopical visualization of effector cell conglomerates.³ We decided, therefore, to use the more tedious, nonradioactive, well accepted trypan blue technique for determination of cell viability and NK cell activity.³

Besides the patient controls, our study gives many internal controls for correct trypan blue uptake. The increase of NK cell activity due to interferon and the differences in NK lymphocytes are well-recognized with the trypan blue exclusion test. It may be true that dead cells which have taken up trypan blue for *longer* periods may faint, but as we are comparing the counts of viable cells before and after the incubation, a theoretical fault of an inaccurate dead cell count is irrelevant.

With regard to cell swelling, we were never able to observe any cell swelling of the K562 cells, even though they were cocultured with other cells during the very *short* incubation period with trypan blue. It is, however, very well-known that the phenomenon described by Drs. Ghoneum, Gill, and Perry occurs if any traces of serum are present, leading to cell swelling and to a reduced trypan blue uptake.⁴

Respectfully,
Thomas P. U. Wustrow, MD
Hans Peter Zenner, MD

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Dear Editor:

We recently read with interest the letter in your column from Dr. G. J. Spector.¹ We would like to share our experience using botulinum toxin (BOTOX) for the treatment of "spastic dysphonia" as part of a trial of toxin injections for the treatment of other cranial dystonias.² The clinical syndrome of "spastic dysphonia" includes patients with focal dystonia of the larynx, in addition to patients with essential tremor and other movement disorders of the larynx.³

We agree that the options for treatment of dystonic dysphonia are limited. We have used pharmacotherapy with limited success. Aronson⁴ reported a 64% long-term failure rate for recurrent laryngeal nerve section. We suggest that paralysis of one cord results in hyperfunction of the other cord, and symptoms return, or become worse.

We have used local injections of BOTOX for the treatment of dystonic muscles in 97 patients: blepharospasm (46), torticollis (29), oromandibular dys-

tonia (4), limb dystonia (3), laryngeal dystonia (3), tongue dystonia (1), and hemifacial spasm (2). In contrast to surgery, in which interruption of nerves to the involved muscles may result in complete and permanent weakness, the approach of chemically weakening sustained contractions has advantages. The injections are performed with the patient awake and titration of dose is performed in order to achieve the desired degree of weakness. If too much local weakness is induced, strength gradually returns. Finally, the procedure is acceptable to the patients, and most were satisfied with the result.

In our series, there have been no persistent clinically significant side effects; patients did not complain of significant pain, only minor discomfort. Two thirds of all patients experienced moderate-to-marked relief of symptoms. Most patients returned for repeat therapy, as this was preferred to pharmacotherapy or surgery. However, approximately one third of the blepharospasm and torticollis patients experienced either mild, or no benefit, and were considered clinical failures.

We have documented electrophysiologic and serologic evidence of distant effects of BOTOX injections. Jitter, an electromyographic parameter denoting impaired neuromuscular transmission, has been seen in patients treated for blepharospasm³ and torticollis.⁴ In our series, jitter has persisted for 9 months after last injection in one patient treated for torticollis, suggesting incomplete recovery of neuromuscular function. However, although these abnormalities are detected in the laboratory, the patients are unaware of any distant effect. In addition, using an in-vivo mouse assay, we have seen antibody production in two torticollis patients. These observations imply that, in this setting, the toxin leaks into the circulation, affects distant muscles, and is immunogenic.

Relevant to your readers are three patients with adductor dystonic dysphonia who participated in a clinical trial of treatment with local injections of BOTOX; drug therapy was of no benefit in two. Written informed consent was obtained. Injections were made through an electromyography/injection needle according to the method described.³ Unilateral injections did not result in clinical improvement; bilateral injections have effected significant clinical improvement in all; the longest period of improvement is 6 months in a follow-up of 6 to 18 months. The procedure is tolerated well; patients typically experience a transient breathy aphonia and slight aspiration lasting 3 days.

The optimal therapeutic approach for treatment of cranial dystonias is elusive. Local injections of BOTOX have helped many patients with disabling dystonias. Additional clinical and laboratory research is needed to further define the doses required for each region treated, pathophysiology of the response, and potential for side effects. Unfor-

tunately, the toxin is presently not available because the manufacturer is having difficulty obtaining product liability insurance.

Most sincerely yours,

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Dear Editor:

RE: Latissimus Dorsi Myocutaneous-Iliac Bone Flap for Reconstruction of Massive Defects of Mandible and Oral Basis (*LARYNGOSCOPE*, 96:171-177, February, 1986).

The cited article presents an interesting variation of staged reconstruction for major mandibular resection. The technique in general may ultimately prove successful. However, the authors report on two cases; one is described as having "insufficient" connection of the implanted bone and ultimately died of recurrence 9 months after surgery. The second patient never resumed the ability to chew solid foods and died at 14 months.

One of the generally accepted criteria for success in mandibular reconstruction is the resumption of oral alimentation. Another criteria is success at one year following the reconstructive effort. I submit to you that this article should have been rejected for publication as being premature and inadequate in its presentation of data.

Yours truly,

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