Author’s reply to letter by Burns et al.

Author’s reply

In a criticism of our short report characterizing the immunogenicity of hylan G-F 20 in rabbits, Burns et al. make a number of points that are misinformed and fail to view our findings within the framework of the many related preclinical and clinical reports in the literature. The authors attempt to discredit our conclusions that an immunogenic component in hylan may underlie severe acute inflammatory reactions (SAIRs) by pointing out that all hyaluronate-based preparations have residual non-human protein levels that could be immunogenic in humans. However, immunogenicity relates as importantly to quality as to the quantity of the potential immunogen. The authors seem to be unaware of our previously published study in which we describe the details of the immunogenicity testing in rabbits and the source of the pooled sera for the present study. Clearly, there are residual proteins in all hyaluronan preparations, including hylan, but experimental attempts in four separate studies have only demonstrated a response to hylan.

Our working hypothesis, developed to attempt to explain this empirical observation, is that the chemical crosslinking of hylan during its manufacture, a process known to enhance immunogenicity of proteins in general, may qualitatively change the nature of residual contaminants, rendering them immunogenic. Our present short communication adds credence to this assumption and even identifies a unique band that is recognized only by pooled sera from hylan G-F 20-immunized rabbits.

The authors further state that sodium hyaluronate contains the warning on its label that anaphylactoid and allergic reactions have been reported, which is a warning not contained on the hylan label, and cite this as proof that sodium hyaluronate can be immunogenic. Basic principles of pharmaco-vigilance state that postmarketing information included in product labels, based on spontaneous anecdotal reports filed by health professionals or patients regarding products that differ in their global distribution and duration of time in the respective markets, cannot be used to scientifically determine a frequency of an event, or to compare products with respect to the occurrence of a given event. Furthermore, we remind the authors that the hylan G-F 20 label does contain contraindication language that states “Do not administer to patients with known hypersensitivity (allergy) to hyaluronan (sodium hyaluronate) preparations.”

Additional patient information in the hylan G-F 20 labeling implies that prior sensitization or exposure is associated with a higher frequency of reactions: “The occurrence of post-injection effusion may be associated with patient history of effusion, advanced stage of disease and/or the number of injections or treatment courses a patient receives.”

regard to postmarketing information, we should also look at published reports and their temporal relationship to product usage. There have been over 150 million injections of the naturally extracted sodium hyaluronate products administered since 1987 without a single published report of SAIRs being associated with any of those products approved in the US. Within the same timeframe, the numerous reports of SAIRs in the clinical literature, which provide sufficient information to identify the distinct clinical nature of these reactions, have related only to hylan.

The authors incorrectly state that a “more thorough” reading of the article by Puttick et al. indicates that while one patient had chicken-reactive antibodies following treatment with hylan, this patient did not have antibodies to hylan. In that report, the authors actually stated that this patient “had significant antibody titers to hylan and to chicken serum proteins...Serum antibodies to intact hyaluronan were not found.” This finding, while obviously uncontrolled, was certainly suggestive and helped form the groundwork for our corroborating investigations as well as others.

Burns et al. criticized our experimental methods, stating that it would be useful for interpretation to understand whether these were the same immunized animals as in our previous study. Yet in our report, we clearly stated that Western blots were performed “using the rabbit antisera from rabbits immunized with various sources of hyaluronans as previously described”; in other words, sera from the same animals were used. Pooling of antisera for biochemical evaluations is not uncommon, and we think that in showing enzyme linked immunosorbent assay (ELISA) results from both individual rabbits and our sera pool, we have accomplished our aim to demonstrate that the pooled sera used for the Western analysis exhibited the same performance in the ELISA that we described in our previous publication. Finally, we fail to understand how identification of the migration of hyaluronidase or other contaminants in the gel system would change the interpretation of our results, as these remained the same across blots developed with Crude Rooster Comb (CRC)-, hylan-, and sodium hyaluronate-specific sera. In addition, the authors state that the faint reactivity of the 6–8 kDa band compared with the strong reactivity of the CRC antisera does not appear to correlate with the ELISA reactivity. We find this observation to be irrelevant, as there are many immunogenic species present in the CRC preparation that are not present in the finished hyaluronate products, which likely contributed to the CRC anti-hylan immune response; it is unlikely the band seen in this region on the CRC blot represents only our species of interest. In response to the authors’ assertion that a demonstration of hylan antisera reactivity with a hylan Western blot would be more convincing, we point out that the amount of starting hylan product per gel lane necessary to detect a single species on Western blot imposes technical limitations. We believe that purification of this component from hylan will be

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necessary to carry out the proper permutation of the study and, because the cost of hylan G-F 20 is prohibitive, we would encourage the company to conduct such studies as a commitment to further explore this safety concern.

The authors state that it is not credible to imply that no immunogenic potential exists for an implantable material. We never made nor intended to make such a statement. We stated only that a difference in immunogenicity may underlie the SAIRs that have been documented in the literature. It is important to interpret our findings within the framework of building published evidence from numerous laboratories on this topic. There are now 16 published reports of SAIRs to hylan, reviewed in Hamburger et al.\(^\text{1}\) and Goldberg and Coutts\(^\text{4}\) or described in four newer reports\(^\text{5}-\text{12}\); none have associated these reactions with sodium hyaluronate. There have also been four clinical reports regarding chronic granulomatous reactions following hylan G-F 20 injections, the most recent of which was initially diagnosed as a sarcoma, and subsequently termed a ‘pseudosarcoma’\(^\text{13}\). The preclinical data leading to similar conclusions to those we have made are particularly compelling: these reports have demonstrated that hylan can elicit antibody responses, passive cutaneous anaphylaxis, inflammatory infiltrates after repeat exposure, and granulomatous reactions in guinea pigs, mice and rabbits—whereas two comparator, naturally derived sodium hyaluronates elicited no discernible reaction\(^\text{1}-\text{4}\). Our recent work was a logical extension to further characterize the possible cause of this response. We acknowledge the preliminary nature of the work, but think that there are sufficient data indicating a difference in how these two products are recognized by animals.

Further work, now ongoing, is necessary to identify and further characterize this species and to evaluate if such a target plays a role in the reactions seen in patients. We find it disturbing that the authors’ response as representatives of the company that manufactures and distributes hylan G-F 20 was entirely antagonistic, and ignored the body of clinical and preclinical literature documenting the occurrence of these reactions to hylan G-F 20. In light of the present climate regarding patient safety as it pertains to the current oral therapies utilized for chronic pain management of OA, we hoped such observations would have been embraced by the company, and a commitment made to follow-up on findings that represent a legitimate safety concern. In this way, all of us can best serve the safety and needs of our patients.

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References