Immunology of Injectable Collagen in Human Subjects

FRANK DELUSTRO, Ph.D.
VICTORIA MACKINNON, B.S.
NEIL A. SWANSON, M.D.

ARTICLE

Abstract. Associated with the use of Zyderm Collagen Implant (ZCI) for soft tissue augmentation, the rate of localized hypersensitivity reactions to the initial test injection of ZCI range from 3.0–3.5% in the literature, and subsequent reactions to treatment have reportedly ranged from 1.1–5.0%. The inflammatory symptoms to the Collagen Test Implant occur within 72 hours in 2% of those injected, indicating a preexisting sensitivity to bovine collagen in this healthy population. Most adverse treatment reactions follow the first treatment and after injection of <5 ml of collagen. Furthermore, antibodies against collagen in sera of subjects reporting localized symptoms of hypersensitivity at test or treatment sites are specific for bovine interstitial collagens and show no cross-reactivity with human collagens. Thus, immunologic reaction to ZCI results in antibovine collagen antibodies and localized inflammatory symptoms in those few subjects who experience hypersensitivity to test or treatment.

Glutaraldehyde cross-linked Zyplast Implant (ZI), has demonstrated a lower incidence of hypersensitivity reactions than ZCI. In examining patients tested with ZI or treated for intradermal and subdermal indications, we have experienced only 7 hypersensitivity reactions at test sites of ZI, out of 803 tested subjects, and only 3 reactions among 498 treated patients. Therefore, ZI appears to induce a lower incidence of hypersensitivity reactions than ZCI in man.

Frank DeLustro, Ph.D., and Victoria Mackinnon, B.S., are from the Clinical Sciences Section, Collagen Corporation, Palo Alto, California.

Neil A. Swanson, M.D., is from the Department of Dermatology, School of Medicine, University of Michigan, Ann Arbor, Michigan.

Address reprint requests to Neil A. Swanson, M.D., University of Michigan Hospitals, 1500 East Medical Center Drive, Ann Arbor, MI 48109.

INTRODUCTION

The extensive utilization of collagen-based biomaterials for hemostatic devices, sutures, heart valve replacements, and vascular substitutes is based on its strong biocompatibility and weak immunogenicity.[1-3] In the search for an injectable material for augmentation of soft tissue contour defects due to scars, rhytids, and genetic deformities, Knapp et al.[4] demonstrated the successful use of type I collagen—the major natural structural component of the dermis—in such clinical indications. Purified, injectable bovine collagen (ZCI) has proved to be a valuable biomaterial for these indications.[5-7] Clinical use of injectable collagen has demonstrated a low incidence of hypersensitivity reactions to this bovine collagen.[8]

Glutaraldehyde cross-linked collagen has been associated with further reductions in immunogenicity as evidenced by enhanced biocompatibility of xenogeneic implants.[9,10] We have begun to examine injectable cross-linked bovine collagen for soft tissue augmentation in man. These immunologic experiences are summarized here.

MATERIALS AND METHODS

Injectable Collagen

Zyderm Collagen Implant (ZCI) was prepared from bovine hide as previously described[4,5] and was utilized as a sterile, fibrillar suspension in phosphate-buffered saline at pH 7.2 at 35 or 65mg/ml. ZI was also prepared from purified bovine collagen as described in detail by McPherson et al.[11] and used as a 35mg/ml suspension in phosphate-buffered saline at pH 7.2.

ZCI Clinical Use

Data on patients tested intradermally with 0.1 ml ZCI in the volar forearm and treated with ZCI for soft tissue contour defects were gathered from the multicenter clinical studies performed,[8] including 9,427 tested subjects and 5,109 treated patients. Additional data have been gathered on patients treated with ZCI during four years (7/81–6/85) of postmarketing. During this time approximately 200,000 patients were treated with ZCI domestically, as determined by the volume of ZCI sold in the United States. Definitions of adverse reactions have been detailed previously.[8]

ZI Clinical Use

Data presented here on people tested or treated with ZI were gathered in controlled clinical trials conducted under the Investigational Device Exemption rules of the U.S. Food and Drug Administration, and with appropriate Institutional Review Board-approved informed consent from participating subjects. In the Sensitivity Study, human volunteers were injected intradermally with a 0.1-ml test dose of ZI in the volar forearm. The test site was observed for hypersensitivity and sera were obtained both before injection and at intervals detailed in the text. Additional patient data have been obtained from patients under study for the subdermal treatment of keratoses of the feet, intradermal treatment of soft tissue defects, and subdermal treatment of contour irregularities with ZI. Localized hypersensitivity reactions were defined as erythema, induration, and/or pruritus, as well as antibodies against bovine collagen.

Antibody Assays

Radioimmunoassay (RIA) for antibodies against collagen has been described in detail previously.[12]

The Enzyme-Linked Immunosorbent Assay (ELISA) was performed as previously described.[13] In addition to a conjugate of wide antibody class specificity,[13] peroxidase-labeled rabbit antihuman IgG, IgA, or IgM antibody conjugates were utilized to measure class-specific antibody activities. These techniques provide sensitive and specific mechanisms to examine anticollagen humoral immune responses.

RESULTS AND DISCUSSION

Following the extensive use of ZCI for the clinical treatment of dermal contour defects, several publications have appeared [8,14 16] that examine the rate of hypersensitivity to test and treatment (Table 1). The response to test ranges from 3.0% in a study of 9,427 subjects by Cooperman et al.[8] and 3.1% in a study of 7,000 subjects by Castrow and Krull,[14] to 3.5% in an investigation of 300 patients by Kamer and Churukian.[15] Similarly, these studies[8,14,15] have reported treated site adverse reaction rates of 1.1–1.5%, while another report[16] has indicated a higher incidence (5.0%) in 202 patients (Table 1). These differences may be related to the number of patients examined, the specific demographics of the study populations, or the clinical interpretation of symptoms of hypersensitivity.

In a study of reactions to skin test implantation of ZCI,[8] the time of onset of the symptoms provides a useful insight into the presence of sensitivity to bovine collagen in the healthy population. In Figure 1, it can be seen that 70% of the hypersensitivity responses to test implantation (first exposure) with ZCI occur within 72 hours, and the data suggest a preexisting immunity to bovine collagen in these subjects. Of the remaining test site reactions, approximately 10% take place from 3–7 days, and the

TABLE 1
Localized Hypersensitivity Reactions to Zyderm Collagen Implant

Reference	Hypersensitivity Response Following:	Number of Patients Followed	Incidence
Cooperman (1985)[8]	Test Injection	9,427	3.0%
	Treatment	5,109	1.1%
Siegle (1984)[16]	Treatment	202	5.0%
Kamer (1984)[15]	Test Injection	300	3.5%
	Treatment	300	1.3%
Castrow (1983)[14]	Test Injection	7,000	3.1%
	Treatment	7,000	1.5%

remainder develop over approximately 1 month. It is of interest to note the presensitization, presumably through dietary exposure to collagen, of approximately 2% of the prospective patient pool, which represents a healthy population; in addition, antibodies to bovine collagen have been demonstrated in the sera of 1–8% of uninjected, healthy controls.[17,18] The data suggest that those subjects who are a high responder genotype produce antibodies rapidly in vivo to a minimal antigenic exposure, such as through environmental exposure or the Collagen Test Implant, and as these subjects are detected after early exposures, late sensitizations are infrequently encountered.[8,18]

Of the patients who are treated with ZCI, hypersensitivity reactions can occur.[8,14–16] In a multicenter clinical study of 5,109 treated patients,[8] it was found that increasing total volumes of ZCI utilized to treat these patients was not found to result in an increasing incidence of hypersensitivity (Table 2). Hypersensitivity reactions were observed in 1.6% of patients treated with < 5 ml, and exposure to larger volumes did not increase the incidence of sensitization to the implant material (Table 2). These results can be better understood in view of the incidence of hypersensitivity reactions following treatment relative to the number of treatment sessions (Fig. 2). In examining over 852 treatment site reactions in the estimated 200,000 patients treated with ZCI in the United States from July 1981 to June 1985, most hypersensitivity (56%) followed the first treatment with ZCI, and 28% followed the second treatment. These data indicate that most hypersensitivity reactions to bovine collagen take place after the earliest exposures (test and first treatment), and subsequent treatments with ZCI

Inflammatory Symptoms at Test Sites of ZYDERM® Collagen

Onset of Inflammatory Symptoms
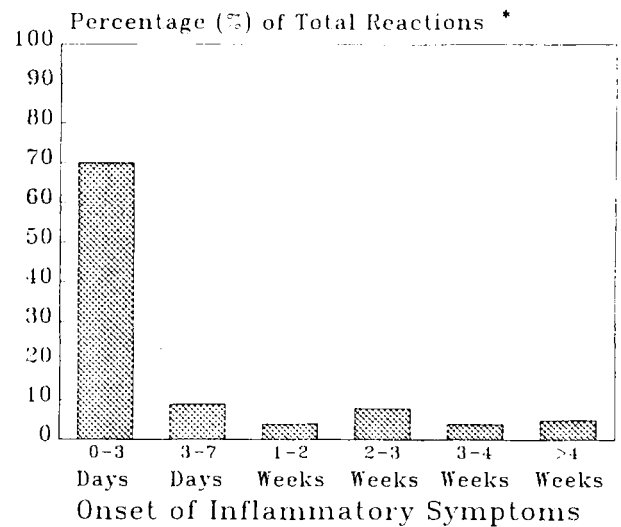
* Responses (n=284) reported to Collagen Corporation during controlled clinical trials with 9427 patients.

FIGURE 1. During a prospective study of 9,427 patients who received an intradermal test injection of ZCI, 284 subjects experienced adverse reactions and the onset of inflammatory symptoms at test sites. The onset times of these reactions were monitored, and the results are expressed as the percentage of the total reactions (n = 284) reported with increasing time following injection.

have significantly diminished potential for eliciting a hypersensitivity response in situ (Fig. 2). Together, these findings (Figs. 1 and 2, Table 2) demonstrate that, in those subjects who display hypersensitivity to bovine collagen, sensitization to ZCI usually takes place after initial exposures to mini-

TABLE 2
Adverse Reactions Against Zyderm Collagen Implant According to Volumes Injected[a]

Total[b] Treatment Volume	Number of Patients Treated	Number of Patients with Adverse Reactions	Incidence of Adverse Reactions
< 5 ml	3,885	62	1.6%
5–9.9 ml	845	4	0.5%
10–29 ml	355	1	0.3%
30–49 ml	19	0	0
50–100 ml	4	0	0
> 100 ml	1	0	0
All	5,109	67[c]	1.3%

[a]Data were collected by the Collagen Corporation during controlled clinical trials with 5,109 treated subjects.[8]
[b]Total amount of ZCI injected per patient during the entire course of treatment.
[c]This group of patients includes subjects (n = 27) who were treated following an unreported positive test reaction.

Treatment Site Reactions To ZYDERM® Collagen Relative to Number of Treatments
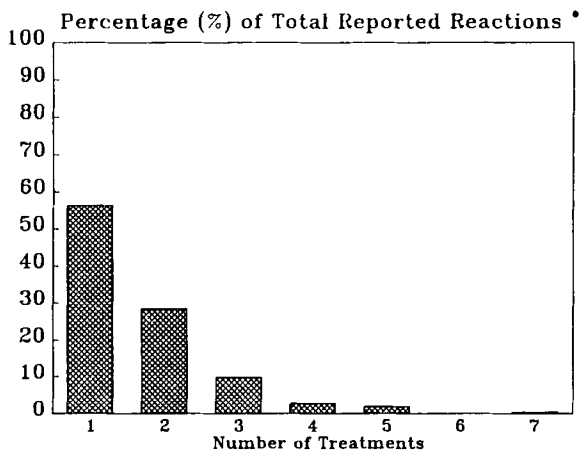
* Responses (n=852) reported to Collagen Corporation from 7/81 to 6/85; an estimated 200,000 patients treated in the U.S.

FIGURE 2. From 7/81 to 6/85, 852 patients reportedly experienced hypersensitivity reactions to ZCI out of a population of approximately 200,000 treated patients. The data are expressed as the percentage of the total reactions (n = 852) occurring after the last reported treatment session.
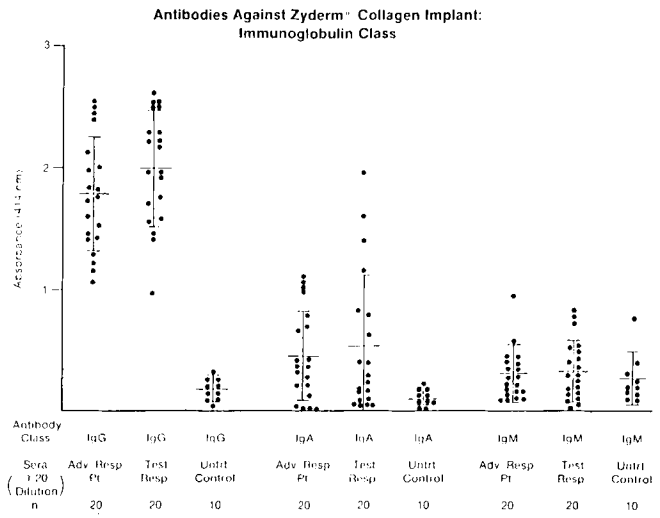

FIGURE 3. Sera from patients experiencing adverse reactions at test sites (Tet Resp.) or treatment sites (Adv. Resp. Pt.), as well as untreated controls (Untrt. Control), were examined in the ELISA for IgG, A, and M antibodies against ZCI. All sera were assayed in duplicate at a 1:20 dilution. Data are expressed as the absorbance values for all sera tested; for each group, the mean absorbance ±SD is indicated by solid bars.

mal volumes of material; repeated treatment with significant volumes of ZCI does not increase the incidence of hypersensitivity.

It has been reported that hypersensitivity to ZCI can be monitored accurately by examining circulating antibodies to bovine collagen.[12,13,16-18] As demonstrated in Table 3, localized hypersensitivity reactions at sites of treatment with ZCI are associated with the detection of circulating antibodies against ZCI as measured by the ELISA or RIA, while systemic reports (such as malaise, arthralgia, and swelling) without localized symptoms have not been found to be associated with detectable antibodies against bovine collagen. However, no sig-

nificant reactivity is observed against human interstitial collagens,[13,18] and only infrequent, weak cross-reaction is observed against guinea pig or rat type I collagen.[13] As previously described,[17] anticollagen antibodies are predominantly IgG and are found to the same extent in sera of patients reacting against a test or treatment with ZCI (Fig. 3). In addition, while IgG is the predominant antibody class observed, sera from some adverse response patients demonstrate circulating IgA anticollagen antibodies (Fig. 3) when compared to control sera from unexposed subjects; IgM antibodies against ZCI are not present. The presence of antibodies to bovine collagen has also been demonstrated in sera

TABLE 3
Development of Antibodies Against Zyderm Collagen Implant in Sera of Patients Experiencing a Localized Treatment Site Response but not with Systemic Complaints

Reaction Site	Number of Patients Examined	Anti-ZCI Antibodies[a]		
		Positive +	Equivocal +/-	Negative -
Treatment Site	93	82[b] (88.2%)	4 (4.3%)	7[b] (7.5%)
Systemic Only	25	0[b] (0%)	3 (12.0%)	22[b] (88.0%)

[a]Sera examined by RIA or ELISA
[b]Chi-squared: p < 0.001

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of patients without symptoms of hypersensitivity after treatment with ZCI but in this case they do not appear to have any clinical significance.[17] Thus, hypersensitivity to ZCI, as measured through circulating anticollagen antibodies, is associated with IgG antibodies that react with sequential and conformational determinants on bovine interstitial collagens and that demonstrate a species specificity for bovine collagen.

Results from studies with glutaraldehyde-treated xenografts and allografts have indicated a minimal host response and prolonged graft survival.[9,10,19-21] These data in animals and human subjects have demonstrated the potential value of cross-linked dermal grafts.[9,10,19] In initial studies with cross-linked ZI in which 246 volunteers received skin tests, no circulating antibodies to bovine collagen were detected at 1 or 2 months after injection as compared to pretreatment control sera (Table 4). Even when subjects were examined at approximately 6 or 12 months following exposure to the test dose, no follow-up serum was found to possess anticollagen antibody activity when compared to matched pretreatment control serum from the same subject (Table 4). Pretreatment and follow-up sera from only one subject displayed significant binding to ZCI. The absence of elevated levels of anticollagen antibodies in the sera of these subjects during the follow-up period suggests a lower level of reactivity than the 3% of subjects who react to a skin test of ZCI (Table 1).

In ongoing clinical investigations with ZI for the intradermal and subdermal treatment of soft tissue defects, the results suggest that the expected incidence of hypersensitivity to both test and treatment

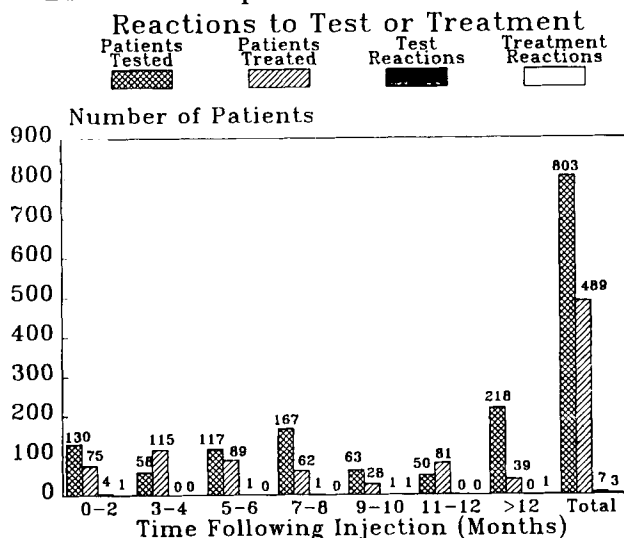ZYPLAST® Implant: Incidence of Adverse Reactions to Test or Treatment

FIGURE 4. Patients received test or treatment injections of Zyplast Implant as described in the text. The total number of patients injected at test or treatment sites is indicated at the last time they were clinically examined to date. Onset of adverse responses is demonstrated by the number of patients having test or treatment site reactions and the time they were first reported. The data are expressed in months following the test injection or following the last treatment implantation for each patient. Numbers above each bar represent the exact number of patients per group.

is significantly lower than that observed with ZCI. In these studies and the sensitivity study previously discussed, we have tested 803 subjects with ZI and followed up 498 patients for >6 months (Fig. 4). The finding of only 7 patients with adverse test site reactions of inflammation and immunity

TABLE 4
Summary of Immunologic Results in Zyplast Implant Sensitivity Studies in Human Volunteers

Group[a]	Sera Source	Number of Patients Examined	ELISA Results[b] Zyplast Implant	ELISA Results[b] Zyderm Collagen
I	Pretreatment	138	0.151 ± 0.072	0.096 ± 0.058
	4-week follow-up	138	0.151 ± 0.077	0.091 ± 0.060
II	Pretreatment	48	0.092 ± 0.061	0.078 ± 0.051
	4-week follow-up	48	0.085 ± 0.056	0.083 ± 0.051
III	Pretreatment	60	0.254 ± 0.113	0.138 ± 0.075
	8-week follow-up	60	0.245 ± 0.123	0.145 ± 0.097
I & II	Pretreatment	131[c]	0.057 ± 0.039	0.020 ± 0.016
	6-week follow-up	132	0.056 ± 0.042	0.021 ± 0.020
I & II	Pretreatment	126[d]	0.143 ± 0.055	0.081 ± 0.089
	12-month follow-ups	131	0.145 ± 0.054	0.085 ± 0.078

[a]Groups I–III represent separately-enrolled study groups at different times.
[b]Data are expressed as the mean absorbance ± SD for 1:20 dilutions of sera.
[c]Pretreatment serum from one subject was not available.
[d]Pretreatment sera from five subjects were not available.

against collagen indicates a reaction rate of 0.9% (7/803) to injection with a test dose of ZI, as compared to the 3–3.5% observed with ZCI (Table 1). As seen in Figure 4, 489 subjects have been treated with ZI, and 210 have been examined at >6 months following exposure. Only 3 adverse treatment reactions with immunity against collagen have been observed, for an incidence to date of 0.6% (3/489), and two of these have occurred at >6 months following treatment (2/210, 1.0%). The current data suggest that the immunogenicity of ZI is reduced below that found with ZCI (Table 1).

CONCLUSION

For many decades, millions of patients have benefited from treatment with collagen-derived sutures, hemostatic agents, vascular prostheses, and injectable fibrillar collagen. The presence of naturally occurring antibodies to collagen has been reported in healthy human control populations[17,23–25] but collagen-derived biomaterials have nonetheless proved to be of great clinical value and of minimal immunogenicity.[1–3] Injectable bovine collagen has provided a valuable clinical alternative for the treatment of dermal contour defects with few adverse events.[4–7]

When these adverse reactions do occur at test or treatment sites, they can be characterized histologically as localized hypersensitivity reactions that demonstrate the local accumulation of a lymphohistiocytic infiltrate.[26] This picture of an immune reaction is accompanied by the detection of circulating antibodies against bovine collagen in 90–100% of these patients.[12,13,17,18] Antibody activity against ZCI is associated clinically with local hypersensitivity reactions, not with systemic complaints (Table 3). These antibodies have been well characterized, and while reacting with native or denatured bovine interstitial collagen, they do not cross-react with human collagen.[12,13,18] Levels of circulating antibodies are similar in patients with hypersensitivity reactions following test or treatment with ZCI. Immunoblotting techniques have demonstrated binding of these antibodies to multiple antigenic determinants in ZCI.[17] Extensive mapping for reactivity with cyanogen bromide peptides of α1(I) and α2(I) chains of bovine type I collagen has verified this heterogeneity and identified α1-CB6 and α2-CB4 as major antigenic determinants on the denatured molecule.[13] This anti-ZCI reaction is best characterized as an immune response against bovine collagen that does not cross-react with collagens of other unrelated species.[8,13,18]

While little direct immunologic data are available on the immunogenicity of cross-linked collagen, the absence of significant clinical or histologic signs of a local hypersensitivity response[9,10,19–21] suggests that there is minimal recognition by the host. Clinical experience with ZI indicates that this assumption is correct.[22] Elson[22] has described hypersensitivity reactions at sites of ZCI only in patients treated with both ZCI and ZI; in these patients, the ZI-injected sites did not react. In this description of clinical experience in over 180 patients treated with ZI, none of the 62 patients treated with ZI alone experienced hypersensitivity reactions. Data from numerous studies in clinical trials and postmarketing use have shown that the incidence of hypersensitivity to ZI compares favorably to ZCI. These results provide further evidence that glutaraldehyde cross-linked (Zyplast) collagen possesses less immunogenicity than its untreated counterpart (ZCI). The potential utility of Zyplast Implant in soft tissue reconstruction is evident.

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