Immunogenicity of bone morphogenetic proteins

A review

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Object. The object of this paper is to review the immunogenicity of bone morphogenetic proteins (BMPs) and to compare the results of the immunogenicity characterization and clinical consequences between recombinant human (rh)BMP-2 and recombinant human osteogenic protein-1 (rhOP-1/BMP-7).

Methods. The immunogenicity of therapeutic proteins and its clinical effects were reviewed. The characteristics of BMPs were also described in terms of immunogenicity. The methods and results of antibody detection in various clinical trials of rhBMP-2 and rhOP-1 were compared, including the most recent studies using a systematic characterization strategy with both a binding assay and bioassay.

Results. Similar to all recombinant human proteins, rhBMPs induce immune responses in a select subgroup of patients. Adverse effects from this response in these patients, however, have not been reported with antibody formation to either rhBMP-2 or rhOP-1. Overall, the incidence of antibody formation was slightly higher in rhOP-1 trials than in rhBMP-2 trials.

Conclusions. Although they occur in a subgroup of patients, the immune responses against rhBMPs have no correlation with any clinical outcome or safety parameter. Clinicians, however, must be aware of the potential complications caused by the immunogenicity of BMPs until more studies clearly elucidate their safety.

(Key Words: bone morphogenetic protein • recombinant human BMP-2 • osteogenic protein-1 • immunogenicity • antibody formation)

Immunogenicity of Therapeutic Proteins

Since insulin became available for clinical use for the first time in the 1920s,56 therapeutic proteins have become an important part of treatment strategy in a diverse range of diseases. Initially, concerns were raised about the immunogenicity of some therapeutic proteins because of their nonhuman origin. However, proteins derived from human sera or tissues and recombinant DNA-derived products that are identical or nearly identical to native human proteins have also proven to be immunogenic.31,68

Immunogenicity of therapeutic proteins may have several clinical consequences caused by the immune reaction itself and the reduction of efficacy of the protein.4,13,54,59,68 First, administration reactions, such as systemic infusion reactions or acute anaphylactic reactions, can be caused by immune complex formation. Immune responses to an exogenous protein can also induce autoimmune to the endogenous form of that protein, leading to long-term detrimental effects.46 Second, an immune response to an administered protein may alter the biological activities of the protein by forming immune complexes, which may influence the pharmacokinetics or pharmacodynamics of the proteins, thus reducing their bioavailability or neutralizing their endogenous counterpart proteins.

The most serious type of immune response that could be associated with a therapeutic protein is a severe hypersensitivity or anaphylactic response.38,51,54,71 Although relatively rare, this response is most frequently associated with nonhuman products, including biopharmaceuticals of bacterial or animal origin such as streptokinase,40 asparaginase,49 murine monoclonal antibodies,45 and aprotinin,15 or products administered in relative high doses such as some monoclonal antibodies. Fortunately, the problem of anaphylactoid reactions to therapeutic proteins has largely been overcome with the development of highly purified products and the advent of recombinant DNA technology.54,59 The current concern
is the potential reduction of efficacy associated with the presence of antibodies.

Formation of antibodies against a therapeutic protein can induce rapid clearance or activity neutralization by binding to its active region. Non-neutralizing antibodies, which bind to the target protein but do not neutralize it, have mostly insignificant clinical effects, although they may abrogate the bioavailability of the protein. These antibodies can even enhance the protein’s biological activity by serving as a carrier that prolongs its circulating half-life. At high titters, neutralizing antibodies can be associated with serious clinical effects by cross-reacting with the native protein. This is especially true when the endogenous counterparts are present at physiologically insignificant levels, or the protein has an essential biological function. The most representative examples of this phenomenon are immune responses to recombinant erythropoietin and thrombopoietin, when patients exhibit pure red cell aplasia and immune-mediated thrombocytopenia, respectively.

A distinction between neutralizing and non-neutralizing antibodies can therefore be clinically important. Assays for antibodies can be grouped into 2 categories: binding assays and bioassays. Binding assays measure antibodies directed toward any part of the molecule, thus detecting both neutralizing and nonneutralizing antibodies. Bioassays demonstrate that antibodies in the serum from patients can neutralize the activity of the therapeutic protein. Although the assessment of neutralizing activity is the best predictor of the impact of elicited antibodies on efficacy, there are no predictors that can substitute at this time for direct clinical assessment. Only clinical studies and careful postmarketing risk management can conclusively elucidate the impact of immunogenicity of therapeutic proteins on the product’s efficacy and safety.

Although there have been several excellent reviews on recommendations and strategies for the assays to detect antibodies against therapeutic proteins, there has also been wide variance in the reported incidence of immunogenicity of a specific protein. This variance is due to the lack of standardization of antibody assays and antibody reference preparations. It is difficult and virtually impossible to compare the results of immunogenicity testing from different clinical trials. The comparison of the immunogenicity of 2 therapeutic proteins cannot be valid unless the 2 products have been evaluated in the same trial using assays with similar sensitivity and specificity. Porter has elegantly described why the analytical exercise to detect and measure antibodies specific for recombinant protein therapeutics is inherently flawed. Many reports of clinical trials actually provide only brief statements on the results of antibody detection without the details of the sensitivity or selectivity of the analytical methods used.

### Immunogenicity of BMPs

Bone morphogenetic proteins are a family of proteins critically important to embryonic development and postnatal bone healing. The initial BMPs were extracted from bone matrix, which contained not only a mixed collection of inductive factors but also several immunogenic matrix factors. Since the first molecular clones of BMPs were characterized, the advent of biotechnology and recombinant DNA technology have led to the production of large quantities of highly purified BMPs. Although the preclinical safety and efficacy of purified bovine BMP extract had been previously investigated, it is these rh-BMPs that are now being evaluated clinically for use in spinal fusion.

Although recombinant human proteins have proven to be generally less immunogenic than those purified from animal tissues, most of them have been shown to induce antibodies. The number of preclinical and clinical studies on the use of rhBMPs and the patients receiving rhBMPs are increasing rapidly, but there have only been a few anecdotal reports on the formation of antibodies. The exact incidence and clinical significance of the reported antibodies have not been clearly demonstrated.

Bone morphogenetic proteins have some characteristic features that distinguish them from other therapeutic proteins regarding immunogenicity. Whereas most therapeutic proteins are used to correct an acquired or genetic deficiency caused by the absence or poor expression of a native protein, rhBMPs were developed to enhance spinal fusion and fracture healing. That is, BMPs are administered locally to address local challenges in bone regeneration and repair, unlike most therapeutic proteins that are administered systemically. The route of administration could be an important factor influencing the incidence of antibody induction, as it is accepted that the subcutaneous and intramuscular administration of proteins may be more immunogenic than intravenous, oral, or aerosolized delivery.

Bone morphogenetic proteins are proteins secreted as soluble factors that have autocrine and paracrine effects. Therapeutic application of BMPs does not appear to cause any systemic toxic effects. This feature may be due to the presence of a highly complex autoregulatory system that blocks BMP action at various levels, and the rapid binding of the active BMP molecule to extracellular factors that modulate BMP activity. Furthermore, some animal studies suggest that rhBMP-2 is so quickly and extensively cleared from the circulation that the systemic presence is negligible.

Hypersensitivity responses may not pose a significant concern in the application of BMPs because they occur infrequently with proteins of human and recombinant origin, particularly in patients with a fully functional endogenous protein counterpart. As previously described, BMPs are not used to replace deficient proteins but to enhance bone regeneration through more intensive recruitment of cells with osteogenic potential. Thus it is unlikely that BMPs would appear as foreign proteins to the patient’s immune system. However, repeat dosing of BMPs has not been recommended in patients in whom antibod-
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ries are detected because of the potential of developing these strong immune reactions.1,4,9,69

The mechanism underlying the induction of antibodies against recombinant human proteins involves a “breach” of immune tolerance for the endogenous protein, whereas antibody responses to nonhuman proteins of plant or bacterial origin is based on the classic reaction to foreign material.3,14,53 How the immune tolerance to self-antigens is broken is not completely understood, but one established way to break tolerance is through the repetitive presentation of self-antigens.50,55 Although the repeated dosing of other recombinant human proteins such as insulin and growth hormone has not compromised their therapeutic activity,48 further studies of the clinical consequences of repeated exposure to rhBMPs should be performed. Carreon et al.4 have recently demonstrated that multiple exposures to rhBMP-2 did not result in clinically detectable allergic reactions, although assays for antibody responses were not performed. As noted by these authors, complications of neck swelling, dysphagia, and bone resorption after the administration of rhBMP-2 have recently been reported.29,39,50,60,61 However, these complications do not appear to be related to the immune responses, but instead are problems related to high dosage and a direct inflammatory response.

The clinical significance of the immune response to exogenously administered rhBMPs remains unclear, and even the exact incidence of nonneutralizing and neutralizing antibody formation against rhBMP-2 and OP-1 has not yet been elucidated. However, based on the large and growing body of literature from clinical studies involving BMP utilization, it is unlikely that antibodies, if present, provoke life-threatening problems in the manner of pure red cell aplasia caused by the cross-reactivity of antibodies to endogenous erythropoietin. One potential exception could be during pregnancy, in which antibodies to rhBMP could cross the placenta and potentially cause devastating effects in the developing fetus. In a preclinical study, rhBMP-2 has been shown to elicit antibodies capable of crossing the placenta.55 Because the influence of maternal antibody formation against rhBMP-2 or OP-1 on human fetal development is unknown, the use of BMPs is contraindicated in women with childbearing potential.49 Preclinical reproductive toxicology studies have been performed in which pregnant female rabbits were hyperimmunized with recombinant OP-1; these rabbits gave birth to normal litters with no observed defects.46

There have been several clinical trials of rhBMPs reporting the results of immunogenicity testing, most of which have demonstrated low antibody formation rates. Antibody production was transient and did not appear to affect the incidence of adverse events, fusion rates, or other clinical outcomes. However, all of the studies simply detected nonneutralizing antibodies by ELISA methods without the description of statistical significance.

Immunogenicity of rhBMP-2

Although not published as separate studies, Poynton and Lane49 have stated that the formation of antibodies against rhBMP-2 and the ACS carrier has been studied after implantation in rats, dogs, and nonhuman primates. Some animals in the canine and nonhuman primate studies had evidence of anti–rhBMP-2 antibodies. Formation of antibodies against bovine type I collagen was also observed in nonhuman primates. In general, all of the immune responses were transient, and the titers were low. No clinical symptoms or allergic reactions developed in any animals with antibodies to rhBMP-2 or bovine type I collagen. The presence of antibodies did not appear to have an impact on the efficacy of rhBMP-2/ACS.

In the first human clinical trial of the use of rhBMP-2/ACS, Boden et al.7 performed serial blood tests in all 11 patients to detect antibodies against rhBMP-2 and bovine type I collagen (Table 1). Antibodies to rhBMP-2 did not develop in any of these patients. Three of the patients had increased anti–bovine collagen antibody titers. None of the patients developed antibodies against human type I collagen, suggesting an absence of cross-reactivity with the bovine collagen. The clinical outcomes of these 3 patients were not affected by the presence of the antibodies. However, the assay methods and the exact time points of the tests were not specified.

In the study of rhBMP-2 in an anterior lumbar interbody fusion model, antibodies to rhBMP-2 developed in only 1 (0.7%) of the 137 patients who received rhBMP-2 and in only 1 (0.8%) of the 124 control patients.5 The incidence of antibody formation to bovine type I collagen was 13.1% for the investigational group and 12.9% in the control group. Antibodies were detected preoperatively and at 3 months after surgery using ELISAs. There was no association between the presence of antibodies and the presence of adverse sequelae or clinical outcome. In the laparoscopic arm of this study,74 the incidence of antibody formation against rhBMP-2 was 0.8%, and 24.8% of the patients were found to have an authentic elevated antibody response to bovine type I collagen (Table 1). No serious adverse sequelae could be associated with these antibody responses. Also, none of the patients in whom antibodies to the bovine collagen carrier were identified had a positive response to human type I collagen. Serological testing was performed 3 months after surgery.

Data from the various FDA-approved investigational device-exempted clinical trials with rhBMP-2 for human spinal fusions have been integrated to provide more meaningful safety and toxicity information.41 A total of 273 investigational patients and 127 control patients were involved in serological testing for the detection of antibodies against rhBMP-2 and type I bovine collagen. Assays were performed preoperatively and 3 months after surgery. Two patients (0.7%) in the investigational group were found to be positive for antibodies to rhBMP-2, and 1 control patient (0.8%) had a positive antibody response to rhBMP-2. Fifty-two patients (19.0%) who received rhBMP-2 had an antibody response to the bovine type I collagen, whereas 16 patients (12.6%) in the control group had a similar response.

The BMP-2 Evaluation in Surgery for Tibial Trauma (BESTT) study group conducted a prospective, controlled, randomized study on the usage of rhBMP-2 for treatment of open tibial fractures.26 Four hundred and fifty patients were randomized into 3 groups: controls,
patients who received an implant containing 0.75 mg/ml of rhBMP-2, and patients who received an implant containing 1.50 mg/ml of rhBMP-2. Assays for antibody responses to rhBMP-2, bovine type I collagen, and human type I collagen were performed at baseline and at 6 and 20 weeks after surgery. One (1%), 3 (2%), and 9 (6%) patients in each group showed the presence of antibodies to BMP-2, respectively. Antibodies to bovine type I collagen developed in 60 patients: 9 (6%), 22 (15%), and 29 (20%) patients in each group, respectively. No patient with antibodies to bovine type I collagen developed antibodies to human type I collagen. There was no relationship between the presence of antibodies and clinical outcome or adverse effects. No patient had a clinical manifestation of an immune response or allergic reaction. The assay method was not specified (Table 1).

Boden and colleagues subsequently reported results from the first clinical study on the use of rhBMP-2 for posterolateral lumbar spine fusion, including the rate of anti-rhBMP-2 antibody formation. A solution containing 20 mg of rhBMP-2 was applied to 10 cm² of biphasic calcium phosphate granules per side of the spine (2 mg/ml) and placed in the decorticated intertransverse process area. Twenty-seven patients were randomized in a 1:2:2 ratio to 3 groups: autogenous iliac crest bone graft, rhBMP-2 with instrumentation, or rhBMP-2 without instrumentation. Twenty-two patients who received rhBMP-2 and 4 patients who received autografts were evaluated for the presence of antibodies. Only 1 of the 22 patients treated using rhBMP-2 (4.5%) exhibited antibody formation against rhBMP-2. No patient in the autograft group was positive for antibodies to human type I collagen. Antibody formation occurred in 3 investigational and 5 control patients. The authors indicated that the bovine collagen antibody formation occurred in 3 investigational and 5 control patients. The authors indicated that the bovine ACS for posterior lumbar interbody fusion. There were 34 patients assigned to the rhBMP-2/ACS group and 33 patients assigned to the autograft group. None of the patients in either group tested positive for antibodies to rhBMP-2 or human type I collagen. Authentic bovine type I collagen antibody formation occurred in 3 investigational and 5 control patients. The authors indicated that the bovine ACS used for the delivery of rhBMP-2 was not the cause of the antibody reaction because there was no difference between the incidence of antibody reactions in the investigational group and the control group. There was also no correlation between Gelfoam sponge use and antibody formation.

Haid et al. evaluated antibodies against rhBMP-2, bovine type I collagen, and human type I collagen before and 3 months after surgery using ELISAs in patients receiving standalone cylindrical-threaded titanium fusion cages with either autogenous bone graft or rhBMP-2/ACS for posterior lumbar interbody fusion. There were 34 patients assigned to the rhBMP-2/ACS group and 33 patients assigned to the autograft group. None of the patients in either group tested positive for antibodies to rhBMP-2 or human type I collagen. Authentic bovine type I collagen antibody formation occurred in 3 investigational and 5 control patients. The authors indicated that the bovine ACS used for the delivery of rhBMP-2 was not the cause of the antibody reaction because there was no difference between the incidence of antibody reactions in the investigational group and the control group. There was also no correlation between Gelfoam sponge use and antibody formation.

Burkus et al. assessed the clinical and radiographic outcomes in patients who received rhBMP-2/ACS with threaded cortical allograft dowels in anterior lumbar spinal surgery. Forty-six patients were enrolled with the use of a 1:1 randomization ratio between study and control groups in the pilot phase. In the pivotal phase, 85 patients were randomized in a 2:1 fashion to the investigational or control group. Blood samples were tested for the detection of antibodies against rhBMP-2 and bovine collagen

### Table 1: Summary of studies of the immunogenicity of rhBMP-2*

<table>
<thead>
<tr>
<th>Authors &amp; Year</th>
<th>Time Point</th>
<th>BMP Group</th>
<th>Antibodies (%)</th>
<th>Control Group</th>
<th>Antibodies (%)</th>
<th>BMP Concentration (mg/ml)</th>
<th>Dose</th>
<th>Assay</th>
</tr>
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<tbody>
<tr>
<td>Boden et al., 2000</td>
<td>NS</td>
<td>11</td>
<td>0 (0.0)</td>
<td>none</td>
<td>none</td>
<td>1.5</td>
<td>1.3 ml/2.6 ml</td>
<td>NS</td>
</tr>
<tr>
<td>Burkus et al., 2002</td>
<td>preop, 3 mos</td>
<td>137</td>
<td>1 (0.7)</td>
<td>124</td>
<td>1 (0.8)</td>
<td>1.5</td>
<td>4.2–8.4 mg</td>
<td>ELISA</td>
</tr>
<tr>
<td>Zdeblick et al., 2001</td>
<td>3 mos</td>
<td>136</td>
<td>1 (0.8)</td>
<td>none</td>
<td>none</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Govender et al., 2002</td>
<td>preop, 6 wks, 20 wks</td>
<td>300</td>
<td>12 (4.0)</td>
<td>150</td>
<td>1 (0.7)</td>
<td>0.75/1.5</td>
<td>6 mg/12 mg</td>
<td>NS</td>
</tr>
<tr>
<td>Boden et al., 2002</td>
<td>NS</td>
<td>22</td>
<td>1 (4.5)</td>
<td>4</td>
<td>0 (0.0)</td>
<td>2.0</td>
<td>20 mg</td>
<td>NS</td>
</tr>
<tr>
<td>Baskin et al., 2003</td>
<td>preop, 3 mos</td>
<td>18</td>
<td>0 (0.0)</td>
<td>15</td>
<td>0 (0.0)</td>
<td>1.5</td>
<td>0.4 ml</td>
<td>NS</td>
</tr>
<tr>
<td>Haid et al., 2004</td>
<td>preop, 3 mos</td>
<td>34</td>
<td>0 (0.0)</td>
<td>33</td>
<td>0 (0.0)</td>
<td>1.5</td>
<td>4.0–8.0 mg</td>
<td>ELISA</td>
</tr>
<tr>
<td>Burkus et al., 2005</td>
<td>preop, 3 mos</td>
<td>78</td>
<td>0 (0.0)</td>
<td>49</td>
<td>0 (0.0)</td>
<td>1.5</td>
<td>8.4–12.0 mg</td>
<td>NS</td>
</tr>
<tr>
<td>Jones et al., 2006</td>
<td>preop, 6 wks, 12 wks, 6 mos</td>
<td>15</td>
<td>0 (0.0)</td>
<td>15</td>
<td>0 (0.0)</td>
<td>1.5</td>
<td>12.0 mg</td>
<td>ELISA</td>
</tr>
</tbody>
</table>

* NS = not specified.
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preoperatively and at 3 months postoperatively. The assay method was not described. Seventy-eight patients in the investigational group and 49 patients in the control group were evaluated for antibody responses. Among those tested, no patient from either group had an antibody response to rhBMP-2. Antibody responses to bovine collagen were positive in 7 patients (9%) in the study group and in 4 patients (8%) in the control group.

The results of testing antibodies against BMP-2 and bovine type I collagen were reported in a recent randomized, controlled trial comparing rhBMP-2 and allograft with autogenous bone graft for reconstruction of diaphyseal tibial fractures. A total of 30 patients were randomly assigned to receive either autogenous iliac crest bone or a combination of allograft and rhBMP-2/ACS (1.5 mg/ml). Antibodies to BMP-2, bovine type I collagen, and human type I collagen were detected by ELISA before surgery and at 6 weeks, 12 weeks, and 6 months after surgery. None of the patients developed antibodies to BMP-2. One patient in the rhBMP-2/allograft group was positive for antibodies against bovine type I collagen at 6 weeks after surgery but not at any subsequent visit. Four patients in the autograft group had increased antibody titers to bovine type I collagen. No antibodies to human type I collagen were detected in patients with anti-bovine type I collagen antibodies.

These studies (Table 1) demonstrate that antibody formation against rhBMP-2 is uncommon and without clinical sequelae. Importantly, no adverse clinical effects have been related to this antibody formation. Immunologically related adverse events, including hypersensitivity responses, have not been reported. Although none of the studies tested for neutralizing antibodies, the incidence of antibody formation itself was so low that the neutralizing activity might also be negligible. These results are consistent with the known characteristics of BMPs regarding the immunogenicity described above.

Most studies detected the presence of antibodies against rhBMP-2 (both nonneutralizing and neutralizing) with ELISA. However, the sensitivity of the ELISA methods used to detect anti–rhBMP-2 antibodies in serum samples from most of these studies has not been described. Moreover, for a number of these studies, antibodies were reported only at the preoperative time point and 1 additional time point after surgery. Given the transient nature of the antibody response, it is possible that the investigators missed the window of appearance of anti–BMP antibodies in the treated subjects. When temporal testing information was provided, antibody formation appeared to be transient.

The reported incidence of anti–rhBMP-2 antibodies in clinical studies is intriguing, given the relatively high incidence of preexisting anti–BMP antibodies in naïve donors recently reported by Sauerborn et al. (unpublished data, 2008). In this study, the authors tested 411 serum samples from naïve donors in anti–BMP-2 and anti–BMP-7 ELISA and found that 9.1% and 6.3% of the samples tested were positive for anti–BMP-7 and anti–BMP-2 antibodies, respectively. One possible explanation for the discrepancy is a difference in sensitivity between the ELISA methods used in the Sauerborn study versus the rhBMP-2 clinical studies. In the Sauerborn study, the ELISA was set based on the recommended 5% false-positive rate and positive samples were confirmed using a competition ELISA, strongly suggesting that the true incidence of preexisting anti–rhBMP-2 antibodies in the population tested is approximately 6%. As mentioned above, no information is available on the sensitivity of the ELISAs used to characterize the immunogenicity of rhBMP-2 in clinical studies.

Immunogenicity of rhBMP-7/OP-1

Geesink et al. first investigated antibody reactions to OP-1 and bovine collagen in 6 patients treated with OP-1 and type I collagen carrier and 6 patients treated with type I collagen alone for a critically sized fibular defect. The serum samples were obtained before surgery and at 1 and 10 weeks after surgery. Two patients who received type I collagen alone developed antibodies against collagen at 10 weeks postoperatively. No patient developed detectable levels of anti–OP-1 antibodies. No reactions were visible at the site of the fibular defect and there were no adverse events reported (Table 2).

In a prospective, randomized clinical trial comparing rhOP-1 to autograft in the treatment of tibial nonunions, all of the 124 patients were screened for antibodies to OP-1 and type I collagen by an ELISA at 1, 2, 3, 6, 9, 12, and 24 months following surgery. The specificity of the response was confirmed with use of Western blot analysis for those patients demonstrating positive anti–OP-1 or anticollegen activity in the screening assay. Anti–OP-1 antibodies developed in 10% of those treated with OP-1, and antibodies against type I collagen were detected in 5% of those patients who received this matrix. All of the antibody responses to OP-1 were transient, and all titers were low. There were no immunologically related adverse events and no correlation between the incidence of anti–OP-1 antibodies and efficacy of the implanted product. All the patients with an anti–OP-1 antibody response were able to experience tibial unions at 24 months after surgery (Table 2).

Additional data suggest an antibody formation rate of 38% in patients treated with rhBMP-7. Titer were low and transient, although antibodies appeared in a high number of patients. There were no systemic adverse events.

A long-term study (> 4 years) has been completed in humans comparing autograft to OP-1 for posterolateral lumbar arthrodesis. Three hundred thirty-six patients were randomized in a 2:1 fashion to receive either OP-1 putty or autogenous iliac crest bone graft. Testing for the presence of anti–OP-1 antibodies was performed in 207 patients of the OP-1 putty group and in 86 patients of the autograft group. Serum samples were collected preoperatively and at regular postoperative intervals and evaluated for the presence of anti–OP-1 antibodies and neutralizing activity using a battery of in vitro binding assays and cell-based bioassays. These assays were developed according to the most recent industry white-paper recommendations with a high sensitivity (300 ng/ml of a positive control anti–OP-1 antibody) and an assay cut-off point set at a 5%
false-positive rate. In addition, highly sensitive neutralizing antibody assays were used to characterize positive anti–OP-1 antibody samples for the presence of neutralizing antibodies. These neutralizing antibodies consisted of primary alkaline phosphatase-based or luciferase-based assays. Potential positive samples for neutralizing antibodies were subsequently confirmed using a secondary quantitative polymerase chain reaction-based assay. Using these highly sensitive assays, 15 patients (8.1%) in the OP-1 putty group and 10 patients (13.3%) in the autograft group were positive for anti–OP-1 antibodies at the preoperative time point. At 24 months postsurgery, 41.0% of the patients receiving OP-1 putty were positive for anti–OP-1 antibodies versus 7.1% of the patients receiving an autograft. Neutralizing antibodies were detected in 25.6% of OP-1 putty-treated patients, but were not detected in any patient at the 24-month postoperative time point. A single patient receiving an autograft (1.2%) also presented with OP-1 neutralizing antibodies. Antibody production peaked in the 6-week to 3-month postoperative time frame and diminished thereafter. Anti–OP-1 antibodies were detected in any patient at the 24-month postoperative time point. A single patient receiving an autograft (1.2%) also presented with OP-1 neutralizing antibodies. Antibody production peaked in the 6-week to 3-month postoperative time frame and diminished thereafter. Anti–OP-1 antibody samples for the presence of neutralizing antibodies. These neutralizing antibodies consisted of primary alkaline phosphatase-based or luciferase-based assays. Potential positive samples for neutralizing antibodies were subsequently confirmed using a secondary quantitative polymerase chain reaction-based assay. Using these highly sensitive assays, 15 patients (8.1%) in the OP-1 putty group and 10 patients (13.3%) in the autograft group were positive for anti–OP-1 antibodies at the preoperative time point. At 24 months postsurgery, 41.0% of the patients receiving OP-1 putty were positive for anti–OP-1 antibodies versus 7.1% of the patients receiving an autograft. Neutralizing antibodies were detected in 25.6% of OP-1 putty-treated patients, but were not detected in any patient at the 24-month postoperative time point. A single patient receiving an autograft (1.2%) also presented with OP-1 neutralizing antibodies. Antibody production peaked in the 6-week to 3-month postoperative time frame and diminished thereafter. Anti–OP-1 antibody status did not correlate with any measure of patient sensitivity of the assay used in these studies is too high or the specificity of the assay. There are no standardized units or positive samples for neutralizing antibodies were subsequently confirmed using a secondary quantitative polymerase chain reaction-based assay. Using these highly sensitive assays, 15 patients (8.1%) in the OP-1 putty group and 10 patients (13.3%) in the autograft group were positive for anti–OP-1 antibodies at the preoperative time point. At 24 months postsurgery, 41.0% of the patients receiving OP-1 putty were positive for anti–OP-1 antibodies versus 7.1% of the patients receiving an autograft. Neutralizing antibodies were detected in 25.6% of OP-1 putty-treated patients, but were not detected in any patient at the 24-month postoperative time point. A single patient receiving an autograft (1.2%) also presented with OP-1 neutralizing antibodies. Antibody production peaked in the 6-week to 3-month postoperative time frame and diminished thereafter. Anti–OP-1 antibody status did not correlate with any measure of patient outcomes or adverse events.

As discussed above for the immunogenicity of rhBMP-2, the high incidence of anti–OP-1 antibodies at the preoperative time point suggests that either the sensitivity of the assay used in these studies is too high or the specificity of the assay. There are no standardized units or positive samples for neutralizing antibodies were subsequently confirmed using a secondary quantitative polymerase chain reaction-based assay. Using these highly sensitive assays, 15 patients (8.1%) in the OP-1 putty group and 10 patients (13.3%) in the autograft group were positive for anti–OP-1 antibodies at the preoperative time point. At 24 months postsurgery, 41.0% of the patients receiving OP-1 putty were positive for anti–OP-1 antibodies versus 7.1% of the patients receiving an autograft. Neutralizing antibodies were detected in 25.6% of OP-1 putty-treated patients, but were not detected in any patient at the 24-month postoperative time point. A single patient receiving an autograft (1.2%) also presented with OP-1 neutralizing antibodies. Antibody production peaked in the 6-week to 3-month postoperative time frame and diminished thereafter. Anti–OP-1 antibody status did not correlate with any measure of patient outcomes or adverse events.

Immune responses, with no apparent effect on the efficacy of the implant itself. According to the previously reported results of immunogenicity testing for bovine type I collagen, it appears that carriers are more likely than rhBMPs to be immunogenic, but antibody production does not appear to interfere with bone formation or bring about adverse events. There is no published information about the difference between the immunogenicity of bovine tendon-derived collagen and bovine bone-derived collagen.

Exposure to exogenous collagen is believed to be primarily dietary in nature. Clinical observations indicate that 2 to 4% of the total population possess an inherent immunity (allergy) to bovine type I collagen.6 Burkus et al.6 have surmised that the relatively high incidence of anti–bovine collagen antibody formation is attributable to a previous exposure through either clinical or environmental means. Given the possibility of a hypersensitivity reaction, many physicians recommend skin tests prior to treatment. However, immune responses to type I bovine collagen used as a bone graft substitute have been limited to reports of elevated levels of circulating antibodies, with no apparent effect on the efficacy of the implant itself.12,43

An additional component of OP-1 putty is carboxymethylcellulose, which is a semisynthetic, anionic water-soluble polymer derived from cellulose, which was chosen to be added to bovine type I collagen to improve handling properties.47 This physiologically inert, biodegradable polymer increases the viscosity and cohesiveness of the delivery vehicle so that the surgeon can easily mold the material. The immunogenicity of carboxymethylcellulose has not been tested in clinical trials of rhBMPs. Some synthetic polymer carriers such as polyactic acid and polyglycolic acid are associated with giant cell inflammatory responses, but these are likely to be foreign-body reactions rather than immune responses.

Comparison of Immunogenicity Between rhBMP-2 and rhOP-1

In general, it is difficult to compare the immunogenicity of recombinant proteins. The incidence of antibody detection is highly dependent on the sensitivity and specificity of the assay. There are no standardized units or pos-
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itive controls for the detection of anti–BMP antibodies. Additionally, the incidence of antibody detection may be influenced by several factors including sample handling, concomitant medications, and underlying disease. For these reasons, comparison of the incidence of antibodies to OP-1 with the incidence of antibodies to rhBMP-2 may be misleading because the tests were conducted in different laboratories using nonstandardized methods. An example of a therapeutic protein with large differences in the reported incidence of antidrug antibodies is interferon-α. Depending on the clinical trial, the incidence of anti-interferon antibodies varied from 0 to 80%. This variation was mostly attributed to differences in the immunogenicity assays used in each clinical study. Given the fact that the reported incidence of preexisting anti–BMP-2 antibodies in naïve human donors is higher than those reported for patients enrolled in some rhBMP-2 trials, the actual presence of anti–BMP-2 antibodies may be higher due to the sensitivities of the assays used to characterize the immune response to this protein. In 2004, Mire-Sluis et al. published in a white paper a series of recommendations for the design of immunoassays. These recommendations are now generally followed by the industry and it is expected that they will contribute to decreasing the disparities between assays used to evaluate the immunogenicity of related therapeutic proteins.

Bone morphogenetic proteins are members of the transforming growth factor-β superfamily, and as such share structural similarities. They can be classified into 3 subclasses according to the derived amino acid sequences. Bone morphogenetic protein-2 and OP-1 belong to different subclasses, and there is an approximately 70% amino acid identity between them. It is still unknown whether the slight structural difference between BMP-2 and OP-1 causes immunological diversity. Perhaps other factors previously mentioned have more profound influences on the immunogenicity of BMPs than the structure itself.

The antibody formation rate of rhOP-1 has been shown to be higher than that of rhBMP-2. The reasons for the high incidence of antibody formation to rhOP-1 are not known. Walker and Wright have proposed that increased clearance time from the circulation may be responsible for the increased antibody formation, but the excretion profiles and kinetic properties of OP-1 were not different from those described for rhBMP-2 in a recently performed experimental study using a rabbit lumbar fusion model. They also recommended routine postoperative serological testing, particularly in patients receiving rhOP-1, for fear that subsequent exposure would induce a significant immune response. No study has been conducted on the safety of repeated administration of OP-1.

**Conclusions**

Concerns about the immunogenicity of therapeutic proteins have been raised since the first protein drug became available. The advent of recombinant DNA technology has made it possible to produce large quantities of therapeutic proteins that are identical or nearly identical to native human proteins, thus eliminating early fears of significant hypersensitivity reactions. However, antibody formation can occur even with these recombinant proteins, and new problems such as pure red cell aplasia have emerged with some proteins. Immunological surveys prior to the development of new protein therapeutics are imperative, and excellent reviews on recommendations and strategies for the assays to detect antibodies against therapeutic proteins have been published. Nonetheless, there is wide variation in the reported incidence of immunogenicity of a specific protein due to the lack of standardization of antibody assays and antibody reference preparations. In addition, immunogenicity is dependent on numerous factors that can be related to patient, treatment, or processing.

Today, two commercially available recombinant human BMPs, rhBMP-2 and rhOP-1, are used to enhance spinal fusion or osteosynthesis and replace autogenous bone graft. Although many patients received rhBMPs including off-label uses, the information about their immunogenicity is scant and the reports on the results of antibody testing are somewhat anecdotal. Immunologically related adverse events have not been observed in the various clinical trials, and antibody formation has never imparted harmful effects on bone formation and clinical outcomes. However, treating physicians have to be aware of the possible clinical consequences caused by the immunogenicity of BMPs, and further studies on safety profiles in pregnant women and fetuses as well as repeated dosing trials are further warranted.

**Disclosure**

Drs. Schellekens and Vaccaro serve as consultants to Stryker Biotech, and Drs. Alaoui-Ismaili and Falb are employees of Stryker Biotech. The remaining authors have no financial relationships to disclose.

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Manuscript submitted July 31, 2008.

Accepted January 23, 2009.

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