# Safety of biologics : current issues and future concerns

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## Introduction

Advances in biotechnology have yielded new options for the management of chronic inflammatory diseases. During the past five years numerous monoclonal antibodies, several cytokines and systemic antisense have all been evaluated in patients with active Crohn's disease. One of these products, infliximab, is now approved for clinical use (1,2). The fundamental potential of the biologics, enhanced efficacy as a result of selective targeting of key inflammatory mediators, seems on the verge of fulfillment. However our knowledge of the human immune system remains rudimentary. Therefore it is important that the safety of these agents is carefully evaluated. This review describes the overall process of evaluating the potential adverse effects of biologic molecules. Specific reference will also be made to the safety profile of infliximab, a monoclonal antibody directed towards tumor necrosis factor.

## Adverse drug reactions and causation

Edwards and Aronson (3) define an adverse drug reaction as "an apparently harmful or unpleasant reaction resulting from an intervention related to the use of medicinal product, which predicts hazard from future administration and warrants prevention or specific treatment, or alteration of the dosage regimen, or withdrawal of the product".

Adverse events due to drugs can result from a number of pharmacological mechanisms including dose related toxicity, idiosyncratic effects and extension effects. The term adverse reaction is a generic descriptor which incorporates all of these mechanisms. In clinical trials it is necessary to distinguish between an adverse effect which is causally related to the study drug and other types of adverse outcomes such as occurrence of a disease exacerbation. This raises the issue of causation. Definitively establishing whether a causal relationship exists between exposure to a new drug and the occurrence of an adverse outcome is often a challenging proposition irrespective of whether an individual patient or a population of patients is under consideration.

Figure 1 shows an approach which can be used to estimate the likelihood that an adverse event is caused by a drug in individual patient (3). If an adverse reaction is sufficiently unusual or occurs as a direct consequence of the drug's biological activity it may be relatively easy to establish a causal relationship to treatment. However obtaining evidence for this linkage is more often difficult if not impossible. Although rechallenge with the

**Certain**
- A clinical event, including a laboratory test abnormality, that occurs in a plausible time relation to drug administration, and which cannot be explained by concurrent disease or other drugs or chemicals
- The response to withdrawal of the drug (dechallenge) should be clinically plausible
- The event must be definitive pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary

**Probable/likely**
- A clinical event, including a laboratory test abnormality, with a reasonable time relation to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal (dechallenge)
- Rechallenge information is not required to fulfil this definition

**Possible**
- A clinical event, including a laboratory test abnormality, with a reasonable time relation to administration of the drug, but which could also be explained by concurrent disease or other drugs or chemicals
- Information on drug withdrawal may be lacking or unclear

**Unlikely**
- A clinical event, including a laboratory test abnormality, with a temporal relation to administration of the drug, which makes a causal relation improbable, and in which other drugs, chemicals, or underlying disease provide plausible explanations

**Conditional/unclassified**
- A clinical event, including a laboratory test abnormality, reported as an adverse reaction, about which more data are essential for a proper assessment or the additional data are being examined

**Unassessable/unclassifiable**
- A report suggesting an adverse reaction that cannot be judged, because information is insufficient or contradictory and cannot be supplemented or verified

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Fig. 1. — Causality assessment of suspected adverse drug reactions

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suspect drug is considered the gold standard for establishing causation, in clinical practice this maneuver is seldom utilized due to ethical or safety considerations. Similar problems exist in evaluating adverse reactions in groups of patients. Figure 2 lists the classic epidemiologic criteria used to establish causation (4).

Various types of studies can be performed to evaluate these criteria. The most valid data comes from randomized controlled trials since these studies incorporate a concurrent control group which minimizes both the potential effects of confounding variables and ascertainment bias. Therefore comparison of event rates between treatment and control groups in Phase II and Phase III efficacy studies are extremely valuable for establishing whether a new drug causes a particular adverse effect. However most serious reactions to drugs are relatively rare. For this reason conventional randomized controlled trials are seldom helpful due to the high risk of a Type II error (inadequate statistical power). Thus, to evaluate many types of adverse events other, sub-experimental epidemiologic studies must be performed.

Fig. 2. — Applying the diagnostic tests for causation*

1. Is there evidence from true experiments to humans ?
2. Is the association strong ?
3. Is the association consistent from study to study ?
4. Is the temporal relationship correct ?
5. Is there a dose-response gradient ?
6. Does the association make epidemiologic sense ?
7. Does the association make biologic sense ?
8. Is the association specific ?
9. Is the association analogous to a previously proven causal association ?

* These diagnostic tests are listed in decreasing order of their importance.
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Furthermore, patients who participate in randomized controlled trials are usually not representative of patients treated in the community. Participants in these studies tend to be healthier and have fewer concomitant medical problems. Therefore analyses based on data from clinical trials may underestimate the frequency of adverse events.

Case reports of unexpected or serious adverse events often initiate questions of causation. This process may begin early in drug development or later in the post-marketing phase. However case reports cannot provide rigorous scientific evidence to definitively establish a causal relationship because of the lack of an appropriate control group and susceptibility to bias. Therefore once an association between the occurrence of an adverse reaction and a specific therapy has been identified more rigorous epidemiologic studies are usually undertaken. Either case-control or cohort studies can be performed. The essential difference between these designs is that in cohort studies rates of adverse events are prospectively compared between individuals exposed to the drug in question and those who are not. Alternatively, the case control study (5) starts by identifying patients with the adverse event of interest and then retrospectively compares drug exposure in affected cases and controls. Both of these approaches have specific advantages and disadvantages. Generally cohort studies are more difficult to perform but yield more rigorous results. The most important advantage of the case control design is that it can be used to evaluate uncommon adverse events. For example, consider the possibility that a new drug is associated with the development of lymphoma. The background incidence of this condition in a population is approximately one in one hundred million. To detect a statistically significant threefold increase in incidence due to a new drug would involve following 750,000 exposed and nonexposed individuals for a period of one year. In this situation the case control design is considerably more efficient since it starts from previously identified cases of the disease. Both of these designs are sensitive to biases (6). However in many situations data obtained from case control, cohort studies or even case reports form the basis for important policy decisions regarding drug safety. In making these decisions a balance must be struck between the severity of the adverse drug reaction, the strength of evidence in support of a causal relationship and the benefits of the drug in question. Sometimes very weak evidence is sufficient to cause a drug to be withdrawn from the market. A recent example is the case of the prokinetic drug, alosetron hydrochloride. Reports linking this agent to ischemic colitis resulted in the United States Food and Drug Administration asking the manufacturer to withdraw the product (7). This decision was made despite lack of evidence from either case control or cohort studies to confirm an association. Which probably reflects the perception of the regulators that the benefits of the drug, which was used to relieve symptoms in patients with irritable bowel syndrome, did not justify the potential, unproven, risk that the drug might rarely cause a serious medical condition. In contrast consider that case reports have suggested that treatment with infliximab might cause lymphoma (8). Despite this issue infliximab has been approved for use in most because the severe and debilitating nature of Crohn's disease justifies the potential risk associated with therapy.

## Safety and biologics : General issues

Safety concerns related to biologics can be classified into four general categories.

First, a possibility exists that infectious agents, originating from the cell lines used in the manufacturing process, might be transmitted to patients (9). In response to this concern numerous safeguards have been imple-

mented to minimize the possibility of viral contamination. For monoclonal antibody production the hybridoma cell line, the cell bank and the final product are all extensively screened for known viruses. Quality control is maintained on this process through the performance of "spiking" experiments in which an aliquot of cells contaminated with a known virus is assayed at regular intervals. To date no evidence exists that any patient has developed infection as a result of treatment with a biopharmaceutical. However previous unfortunate experiences with gamma globulin (10) and vaccines (11) mandates that great care be taken to monitor the manufacturing process using the best available technologies.

The second concern relates to the potential immunogenicity of biologicals. The likelihood that a given product will produce a host immune response is directly related to its "foreignness". Thus murine monoclonal antibodies frequently cause sensitization whereas recombinant human cytokines receptors rarely invoke an immune response. Sensitization can lead to several negative consequences for the patient. First antibody formation can produce either acute allergic reactions, mediated by IgE, or serum sickness due to immune complex formation. Although these adverse events are usually not life-threatening they may produce significant morbidity. Furthermore, antibody formation may result in loss of efficacy if the specificity is directed towards the component of the drug which interacts with the receptor. Several approaches have been used to reduce the possibility of sensitization. In transplantation and rheumatoid arthritis coadministration of other immunosuppressive drugs reduces the risk of sensitization to therapeutic antibodies. Logically, another possibility is to "humanize" the biopharmaceutical using sophisticated molecular techniques. The first generation of drugs derived by this technique were chimeric antibodies-murine/human hybrids-such as infliximab. These molecules are much less likely to cause sensitization than murine monoclonals. More recently the technique of complementarity determining region grafting has been utilized to produce molecule such as CDP 571 (12), a monoclonal antibody directed towards TNF which has only six percent residual murine amino acid residues. Finally advances in technology now allow the production of wholly humanized monoclonal antibodies (13). This should result in even lower risk of sensitization. However even the most sophisticated technology is unlikely to completely eliminate the problem of antibody information against biopharmaceuticals. For example approximately 30 percent of multiple sclerosis patients treated with one type of recombinant beta-interferon develop neutralizing antibodies despite the fact that this molecule is virtually identical to the endogenous human cytokine (14).

The third concern is that treatment with biologics will overly suppress the immune system resulting in an increased risk of opportunistic infection or tumours. Our understanding of the immune system is incomplete. Although the enthusiasm for biologics is based on the belief that more selective "immune modulation" will be safer than broad spectrum agents such as azathioprine and methotrexate little emperic data are available to support this notion. Certainly the experience in transplantation with OKT3 shows that biologics can be associated with an increased risk of both cytomegalovirus infection and lymphoma (15). Although most of the agents currently under development should not cause overt immune suppression it is also plausible that blocking specific mechanisms may cause harm. For example in animal models TNF is important offense against intracellular pathogens (16,17). Could treatment with TNF blockers then predispose patients to infection with tuberculosis, Listeria and fungal pathogens (18) ? Paradoxically, inappropriate stimulation of immune mechanisms may also be an issue with biologics. Two examples are the development of a lupus-like syndrome in patients receiving infliximab (19) and the worsening of multiple sclerosis which has been observed in patients receiving a recombinant p55 TNF receptor (20).

Finally monoclonal antibodies directed towards cell surface proteins can cause cell death either through the activation of the complement system or by inducing apoptosis (21). Several negative consequences may result. Lysis of activated macrophages and T-cells produces a cytokine release syndrome characterized by fever, chills, and organ dysfunction. Depletion of lymphocytes through these mechanisms can also predispose patients to viral infections. Theoretically deletion of clones of effector lymphocytes may result in development of tumors or autoimmune disease as discussed previously. Two approaches have been taken to minimize this problem. Utilization of F (ab)2 fragments of antibody retains the binding capacity of the original molecule while eliminating the components of the parent antibody which are responsible for complement activation. However these immunoglobulin fragments have short half-lives which limits their usefulness as drugs. The recently developed approach of combining the fragments with polyethylene glycol offers a solution to this problem (22). Another tactic is to eliminate functional activity in the $CH_2$ region of the monoclonal through site-specific mutagenesis. Clinical trials evaluating antibodies produce using this technique are currently underway.

Although this array of potential safety problems with biologics seems formidable in practice these agents have been relatively well tolerated and safe in a wide variety of applications. In Crohn's disease the initial experience with infliximab has been consistent with this viewpoint.

## Infliximab : A chimeric antibody directed towards tumor necrosis factor alpha

Infliximab is a genetically engineered immunoglobulin derived from the murine monoclonal antibody cA2 which is directed towards the cytokine tumor necrosis factor alpha (23). The murine constant domains for both

heavy and light chains were replaced by human structural equivalents. The resulting, chimeric, antibody retains the binding capacity of the original murine antibody with reduced potential for sensitization. The molecule has normal glycosylation of the IgG1 subclass. Randomized controlled trials have demonstrated efficacy for this drug in both therapy resistant and fistulizing Crohn's disease (1,24).

## Safety profile of infliximab

Preclinical studies failed to demonstrate important safety concerns (25). Prior to the approval of the drug 14 studies were performed in normal healthy volunteers and patients with sepsis, with rheumatoid arthritis and Crohn's disease. Data from 513 patients with the latter two conditions who participated in randomized controlled trials provides the best data for assessment of the incidence of adverse events in patients with autoimmune disease.

## Infusion reactions

In randomized controlled trials infusion reactions occurred in significantly more infliximab treated patients than those who received placebo (15.9% vs. 6.5%) (26). The typical symptoms included headache, nausea, chest pain, dizziness, flushing and pruritis. These reactions were usually mild and did not require discontinuation of therapy. It is likely that the majority of these reactions occur as a result of nonspecific vascular effects due to administration of a highly concentrated protein solution. Since similar reactions are observed with immunoglobulin infusions used to treat hypogammaglobulinemia. In most cases these reactions can be dealt with by temporarily discontinuing therapy, slowing the rate of infusion, diluting the infusate, or coadministration of antihistamines. More serious infusion reactions, characterized by hypotension in conjunction with one or more of the previously identified symptoms, are more likely to be related to immune mechanisms. In post marketing experience the incidence of these reactions has been estimated to be between 0.1% and 1% (26). Generally, the occurrence of a severe reaction requires cessation of infliximab treatment.

## Other adverse reactions

In clinical trials minor upper respiratory tract infections were more common in patients who received infliximab therapy than placebo (25). Sinusitis and herpes zoster infection also occurred more frequently in patients treated with the active drug. However serious infections were relatively rare and no definite increase in the rate of these events was evident with infliximab therapy. Since infliximab has come into general use individual case reports have described reactivation of tuberculosis in some treated patients. At present it is not clear whether or not the antibody has caused disease reactivation in these individuals. However given the possible adverse effects of blocking TNF on host defenses against intracellular pathogens patients who are candidates for treatment with infliximab should be carefully evaluated for the presence of tuberculosis prior to the initiation of therapy. No evidence suggests that patients who receive the antibody are predisposed to enteric infections. However in the trial by Present and colleagues (2) an increased frequency of new abscess formation was observed in patients with fistulizing Crohn's disease who received active treatment. This may be a consequence of interference with drainage of fistula tracks due to the healing process.

## Antibody formation

### *Antibodies to infliximab*

In patients with Crohn's disease participating in clinical trials, the majority of whom were not treated with concomitant antimetabolite therapy, the overall incidence of antibodies to infliximab was 13%. An increased risk of infusion reactions was noted in these patients (36% vs. 11%) (26). In patients with rheumatoid arthritis coadministration of methotrexate has been documented to reduce the formation of antibody formation. Similarly higher doses of infliximab may also reduce the risk. Whether azathioprine or methotrexate have similar properties in patients with Crohn's disease receiving infliximab is unknown.

Recently cases of serum sickness have been reported in patients who had been retreated with infliximab after a period of greater than two years from the initial infusion. These individuals developed polyarthralgia, skin rash, fever, and myalgias one to two weeks following retreatment. Laboratory investigations revealed high titre antibody to infliximab without hypocomplementemia or an active urine sediment. Treatment with glucocorticoid therapy and discontinuation of the study medication resulted in the resolution of symptoms. Patients who develop this syndrome should not be retreated with infliximab since it is thought to be due to a delayed type hypersensitivity reaction to the drug.

### *Autoantibodies*

Patients treated with infliximab have an increased prevalence of antinuclear antibodies. In the randomized trials a 12% increase in the occurrence of these antibodies was noted from baseline to the completion of follow-up. A minority of patients, approximately 9%, have concomitant antibodies to double-stranded DNA (25). In most cases no clinical consequences of antibody formation occur. However two patients have been described who developed lupus-like symptoms in association with the formation of double-stranded antibodies to DNA. Interestingly, these patients did not demonstrate hypocomplementemia or renal or central nervous system

involvement. Discontinuation of therapy and treatment with prednisone was resulted in resolution of the clinical symptoms of lupus. Antinuclear antibody formation has also been reported with CDP 571 and etanercept therapy suggesting that this phenomenon may be a class effect of anti-TNF agents.

*Neoplasia*

Whether infliximab therapy is associated with an increased risk of malignancy remains unknown. Prior to approval of the drug by regulatory authorities for cases of non-Hodgkin's lymphoma were reported (25). One of these cases occurred in a patient with HIV infection and two cases occurred in patients with rheumatoid arthritis who were also receiving antimetabolite therapy. A single case of gastric lymphoma occurred in a patient with Crohn's disease who received a single infliximab infusion. Given that both HIV and rheumatoid arthritis are associated with the increased risk of lymphoma it is impossible to make any inferences regarding causation on the basis of these data. Following release of the drug several additional cases of lymphoid malignancy have been reported (8). Most recently multiple cases of colon carcinoma and an anal carcinoma have also been described in patients receiving chronic treatment (27). At this point a number of epidemiologic investigations have been initiated to determine whether a causal relation is present. In the interim patients should be informed that small increased risk of a malignancy may exist.

## Summary and future concerns

Our initial safety experience with biologics has been for the most part favorable. Despite theoretical concerns no evidence exists that viral transmission has occurred as a result treatment with these drugs. At the present time immunogenicity is perhaps the greatest clinical problem. However new manufacturing techniques have been developed which will minimize the problems associated with older approaches such as chimeric monoclonal antibodies. Surprisingly opportunistic infection has not proven to be as significant a problem as was anticipated.

Perhaps the greatest concern for the future is that biopharmaceuticals have the potential to interfere with the endogenous immune responses which are responsible for protection against neoplasia and autoimmunity. To further evaluate the extent of this problem appropriate epidemiologic studies must be organized. A key responsibility of clinicians who treat patients with these drugs is to promptly and completely report cases of infection or tumors to appropriate authorities. Ultimately it will require evaluation of large populations of patients over many years to define the relative safety of these agents.

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