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CONCISE COMMUNICATION

Patients receiving etanercept may develop antibodies that interfere with monoclonal antibody laboratory assays

More and more patients are being treated with the drug etanercept (Enbrel; Immunex, Seattle, WA) for rheumatoid arthritis (RA), and we have found evidence that at least a proportion of them will produce antibodies that can interfere with some laboratory assays. Herein we report 2 cases that brought this concern to our attention.

Patient 1 was a middle-aged man with RA who presented to his physician with chest pain 4 months after beginning etanercept treatment. His troponin level (by AxSym methodology; Abbott Laboratories, Abbott Park, IL) was 10.6 ng/ml (normal 0.0–0.4). This prompted an extensive evaluation for myocardial disease, the results of which were negative. Investigation into the methodology behind the assay for troponin prompted retesting of his serum with an altered troponin assay (that included goat serum), and this yielded a normal result (<0.4 ng/ml).

Patient 2, a middle-aged woman with RA, underwent cardiac evaluation for chest discomfort, 4 months after the initiation of etanercept treatment. Her troponin level was initially reported as 1.1 ng/ml, but limited evaluation revealed no evidence of myocardial disease. Repeat testing of the troponin level with the altered assay gave a normal result (<0.4 ng/ml).

Etanercept is a competitive inhibitor of circulating tumor necrosis factor (TNF) (both α and β forms). It consists of the TNF-binding portion of human TNF receptor linked to a fragment of the Fc portion of human IgG1. It is produced via recombinant DNA technology in a Chinese hamster cell line. The IgG1 Fc portion used to link these binding sites contains part of the normal human Fc structure, but not all of it. This molecule is then purified; its biologic action is to bind to soluble TNF and thereby not allow cell surface receptors for TNF to be activated (1–3).

Formation of antibodies to the etanercept molecule has been noted in ~16% of patients taking the drug (2). These antibodies have been non-neutralizing and have apparently not interfered with the drug's actions. In addition, antibodies to double-stranded DNA have been detected in ~15% of treated patients, and antinuclear antibodies in 11%. The significance of these antibodies is presently unknown.

It is certainly not surprising to suspect that this molecule may produce anti-animal antibodies as well. One theory is that this could be the result of minor contamination with hamster proteins from the cell lysate, or perhaps from unintended addition of hamster protein fragments into the molecule itself since it has been generated from the DNA of a host hamster cell. Presumably, one would then have to postulate cross-reactive immunogenicity. It is recognized that any foreign protein can trigger an antibody response. Non-iatrogenic causes of anti-animal antibodies include such mundane activities as owning an animal or ingesting dietary animal antigens (4–6).

The problem arises when such anti–animal protein antibodies (antibodies directed to antigens on proteins from another species) interfere with monoclonal antibody assays that rely on animal-derived monoclonal antibodies with which they will react (7–9). In the 2 cases described herein, mouse anti-human troponin was used as part of the detection system in the troponin assay, and any human anti-mouse antibody could conceivably cross-react with it. This is the most likely mechanism, but it has not been proven. Clearly, however, false-positive elevations in troponin levels occurred in these patients. The diagnostic laboratory that manufactures the assay recognized a possible interference with heterophile antibodies and enhanced the assay by adding goat protein to the conjugate (10). This has eliminated the interference noted above, although the exact component of the goat serum that is the active agent in producing this effect has not been fully characterized.

The application of animal-derived monoclonal antibodies for diagnostic use in clinical samples is increasing. As well, "biologic" agents are being increasingly developed for medical therapeutics. The interface of these molecules is bound to result in interactions that may disrupt animal monoclonal detection systems. A partial list of commercially available assays that involve mouse monoclonal antisera includes assays for creatine kinase–MB fraction, β–human chorionic gonadotropin, folate, vancomycin, thyroid-stimulating hormone, and T4. The clinician should be aware of these possibilities when ordering tests and interpreting results.

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