Sub-Nanogram Detection of RDX Explosive by Monoclonal Antibodies

David O. Ulaeto, Alistair P. Hutchinson, and Stephen Nicklin

Polyclonal and monoclonal antibodies were raised to protein carrier molecules haptenized with RDX, a major component of many plastic explosives including Semtex. Sera from immunized mice detected RDX protein conjugates in standard ELISA. Clonally purified monoclonal antibodies had detection limits in the sub-ng/mL range for underivatized RDX in competition ELISA. The monoclonal antibodies are not dependent on the presence of taggants added during the manufacturing process, and are likely to have utility in the detection of any explosive containing RDX, or RDX contamination of environmental sites.

Introduction

RDX is an explosive chemical(1) that is one of the major components of plastic explosives including Semtex. A high affinity monoclonal antibody specifically recognizing it would have useful properties in the detection of trace amounts of RDX-based explosives and related compounds. RDX is a reactive hapten that, because of its small size, is unable to directly crosslink immunoglobulin molecules on the surface of RDX-specific B cells. Because it is not a protein, it is also unable to stimulate T cell help.(2) In order to generate a high affinity antibody to RDX, it is necessary to chemically conjugate the hapten to a large protein molecule that is capable of eliciting T cell help.

Before the hapten can be conjugated to a protein it must be chemically derivatized to enable it to bind covalently to the protein molecule.(3,4) This process alters the structure of the hapten, raising the possibility that an antibody that recognizes the derivatized hapten may not in fact recognize underivatized pure RDX. In addition, some derivatives possess a relatively long side chain that is not found in the underivatized hapten. Because of the small size of the hapten itself, antibodies raised to the derivative may include portions of the derivative side chain as recognition features, leading to a failure to recognize the hapten with sufficiently high affinity in the absence of the side chain.

Materials and Methods

To avoid the above scenarios, two different derivatives of RDX were used in this project, RDX12, which has a relatively long derivative side chain, and RDX14, which has a very short derivative side chain (Fig. 1). RDX14 conjugates were used for immunizations of 5- to 8-week-old female BALB/c mice, and an RDX12 conjugate was used for screening of sera and hybridoma supernatants. The rationale for this is that only antibodies reactive with both derivatives will be selected, and the only features in common between the derivatives are those that are also shared by underivatized RDX. To further optimize the protocol, the derivatized hapten were conjugated to different carrier proteins, keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA), to ensure that carrier protein determinants were not part of the epitopes recognized by expanded antibodies. KLH-RDX14 conjugate emulsified in complete Freund’s adjuvant (CFA) was used for all primary immunizations; but the mice were also simultaneously immunized with unconjugated BSA, emulsified in CFA, from a separate syringe at a separate site. Booster immunizations used a BSA-RDX14 conjugate emulsified in incomplete Freund’s adjuvant. Hybridomas were generated by standard techniques using the X63-AG8 plasmacytoma fusion partner.(2,5)

Results and Discussion

Initial screens of sera and hybridoma supernatants were undertaken by direct ELISA against RDX12 conjugated to chicken gamma globulin (CGG). CGG was used as a carrier protein as a further precaution against selection of antibodies for which the carrier protein contributed to the recognized epitope. Serum from immunized animals was able to recognize CGG-RDX12 in direct ELISA at dilutions in excess of...
1:1000 (Fig. 2A), indicating that the immunization was successful and hybridoma fusions could be undertaken with a high probability of success. After selection and expansion of hybridomas reactive with CGG-RDX12, further screening was undertaken using competition ELISA where recognition of CGG-RDX12 was competed with underivatized, unconjugated RDX. RDX efficiently competed with CGG-RDX12 for antibody binding, demonstrating specificity for RDX rather than a fortuitous epitope involving carrier protein or derivatization side chain moieties. The limit of detection for RDX was as low as 0.3 ng/mL for the best performing antibodies (Fig. 2B).
Since the early 1990s, it has been an international requirement for explosives to include a volatile taggant that is added during manufacturing. These make a major contribution to detection of bombs, landmines, and explosive caches by sniffer dogs and machine-based detection systems. The antibodies we describe here provide a useful complementary tool to the use of taggants, because they are able to directly and specifically bind RDX, irrespective of the presence of taggant. This has the potential to allow detection and identification of trace amounts of RDX-containing explosives for screening, forensic, or environmental analysis. Several technologies are available for the incorporation of antibodies into optical- and/or resonance-based sensors, which can import the sensitivity and specificity of the antibodies into extant and future sensor and detection technology.

Author Disclosure Statement

The authors have no financial conflicts to disclose.

References

1. Dobratz B: LLNL Explosive Handbook, UCRL-52997. L. Lawrence Livermore National Laboratory, CA, 1981.
2. Harlow E, and Lane D: Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988.
3. Landsteiner K, and Jacobs J: Studies on the sensitization of animals with simple chemical compounds. J Exp Med 1935; 61:643–656.
4. Landsteiner K, and Jacobs J: Studies on the sensitization of animals with simple chemical compounds. II. J Exp Med 1936;64:625–639.
5. Kearney JF, Radbruch A, Liesegang B, and Rajewsky K: A new mouse myeloma cell line that has lost immunoglobulin expression but permits the construction of antibody-secreting hybrid cell lines. J Immunol 1979;123:1548–1550.
6. Marking of Plastic Explosives for the Purpose of Detection convention, Montreal, Canada, March 1991 (ICAO Doc 9571). ICAO (ed). http://www.icao.int/secretariat/legal/Administrative%20Packages/mex_en.pdf
7. Blackburn GM, Beadham IG, Adams H, Hutchinson AP, and Nicklin S: Synthesis of haptens and their protein conjugates for immunological determination of nitrate esters and nitramines. J Chem Soc Perkin Trans 2000;1:225–230.