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The T-cell-dependent antibody response assay in nonclinical studies of pharmaceuticals and chemicals: Study design, data analysis, interpretation

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ABSTRACT

The T-cell-dependent antibody response (TDAR) assay is a measure of immune function that is dependent upon the effectiveness of multiple immune processes, including antigen uptake and presentation, T cell help, B cell activation, and antibody production. It is used for risk and safety assessments, in conjunction with other toxicologic assessments, by the chemical and pharmaceutical industries, and research and regulatory agencies. It is also employed to evaluate investigational drug efficacy in animal pharmacology studies, provide evidence of biological impact in clinical trials, and evaluate immune function in patients with primary or secondary immunodeficiency diseases. Various immunization schemes, analytical methods, approaches to data analysis, and data interpretations are in use. This manuscript summarizes some recommended practices for the conduct and interpretation of the assay in animal studies.

1. Introduction

The kinetics and magnitude of the antigen-specific antibody response following immunization with a T-dependent antigen – the T-cell-dependent antibody response (TDAR) – is used to assess immune function. Antigens are referred to as T-dependent when B lymphocytes require T cell help in order to elicit an optimal antigen-specific antibody response, in contrast to a T-independent antigen, such as a polysaccharide, that can elicit an antibody response without T cell help. Several days after immunization with a novel (neo) T-dependent antigen, antigen-specific antibodies, primarily of the immunoglobulin (Ig) M isotype, are generated and released into the circulation by B cells and plasma cells, i.e., terminally-differentiated B cells (Ladics, 2007b). Antigen-specific

Abbreviations: APC, antigen presenting cells; AUC, area under the curve; CP, cut point; ECL, electrochemiluminescence; ELISA, enzyme-linked immunosorbent assay; EPA, Environmental Protection Agency; GLP, good laboratory practice; HMW, high molecular weight; HBsAg, Hepatitis B surface antigen; ICH, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; Ig, immunoglobulin; KLH, keyhole limpet hemocyanin; NHP, non-human primates; NSB, non-specific binding; PIDD, primary immunodeficiency diseases; PFC, plaque-forming cell; SCID, Severe Combined Immune Deficiencies; SRBC, sheep red blood cell; TDAR, T-cell dependent antibody response; TT, tetanus toxoid; US, United States.

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antibodies of other isotypes (e.g., IgG) and of higher affinity are subsequently released following antibody class switching and somatic hypermutation, respectively (Murphy, 2011). An antigen produces either a primary response (neoantigen) or a secondary response (recall antigen) when the same antigen is used in a second/repeated immunization. The antigen-specific antibody response to a recall antigen is primarily of an isotype other than IgM, is of increased magnitude and occurs with faster kinetics due to immune memory (anamnestic response). Experimental evaluation of antigen-specific antibody-forming cells or circulating antibodies is used to quantify the humoral (antibody) immune response to the antigen.

Multiple immune cell types and functions are required to induce a TDAR (Fig. 1). Immune cell types involved in the TDAR include antigen-presenting cells (APCs; primarily dendritic cells or macrophages), naïve and activated CD4+ T cells and T-follicular helper (T_{FH}) cells, B cells, and plasma cells. Cellular functions required include antigen processing and presentation, differentiation, upregulation of cell surface receptors, secretion of cytokines, somatic hypermutation and immunoglobulin class (isotype) switching. The various immune cells involved must communicate through receptor/ligand and receptor/cytokine interactions (Deenick, 2011). Because an optimal TDAR encompasses coordinated immunological efforts, the TDAR has emerged as a widely used method for assessing immune function in safety assessment studies for environmental chemicals, and both small and large molecule (biologics) pharmaceuticals. It is also employed to evaluate efficacy in nonclinical pharmacology studies and to measure immune function following treatment with pharmaceuticals in the clinical setting (Bingham, 2010; Brodmerkel, 2010; Struijk, 2010), or to evaluate immunodeficiencies (Kuijpers, 1997).

In the late 1980s and early 1990s, the TDAR to sheep red blood cells (SRBC) was extensively evaluated by the National Toxicology Program and was found to be useful for identifying immunosuppressive chemicals in nonclinical studies (Luster, 1992). The assay was based on anti-SRBC antibody (IgM) production by splenocytes (antibody-forming cells) in a semi-solid agar matrix causing the lysis of surrounding SRBC in the presence of complement and was known as the 'plaque-forming cell (PFC) assay', the 'plaque assay' or simply the 'SRBC assay' (Ladics, 2007a,b; White et al., 2010). The predictive value of the same assay was evaluated for the nonclinical safety testing of small molecule pharmaceuticals (Lebrec, 1994) and adapted to evaluate both primary (IgM) and secondary (IgG) responses (Holsapple, 1995). Other T-dependent antigens such as keyhole limpet hemocyanin (KLH) and tetanus toxoid (TT) were subsequently introduced as alternatives to SRBC in the nonclinical testing of xenobiotics.

In response to a recommendation to include the specific evaluation of immunotoxicity as part of the hazard assessment process, in 1998 the United States Environmental Protection Agency (EPA) finalized an immunotoxicity test guideline (OPPTS 870.7800) (US EPA, 1998). In 2007, the assessment of immunotoxicity became a part of the required studies in the revised Toxicology Data Requirements for pesticide registration for food and non-food uses. The intent of the EPA immunotoxicity guidance is to provide information on the ability of a test chemical to suppress the immune system. One of the core requirements under this guideline is the conduct of a TDAR with SRBC as the antigen. More specifically, rats and/or mice are exposed to the test and control substances for at least 28 days and immunized by intravenous or intraperitoneal injection with SRBC approximately 4–6 days prior to the end of the exposure, depending on the immunization route and assay approach (i.e., splenic PFC vs. serum enzyme linked immunosorbent assay [ELISA]) (Ladics, 2007b). At the end of the exposure period, either the PFC assay (Jerne and Nordin, 1963) or an ELISA (Temple, 1993) is performed to determine the effects of the test substance on the primary splenic anti-SRBC IgM response or serum anti-SRBC IgM levels, respectively. Currently the EPA is the only chemicals regulatory agency to have specific immunotoxicity testing requirements although the Organization for Economic Cooperation and

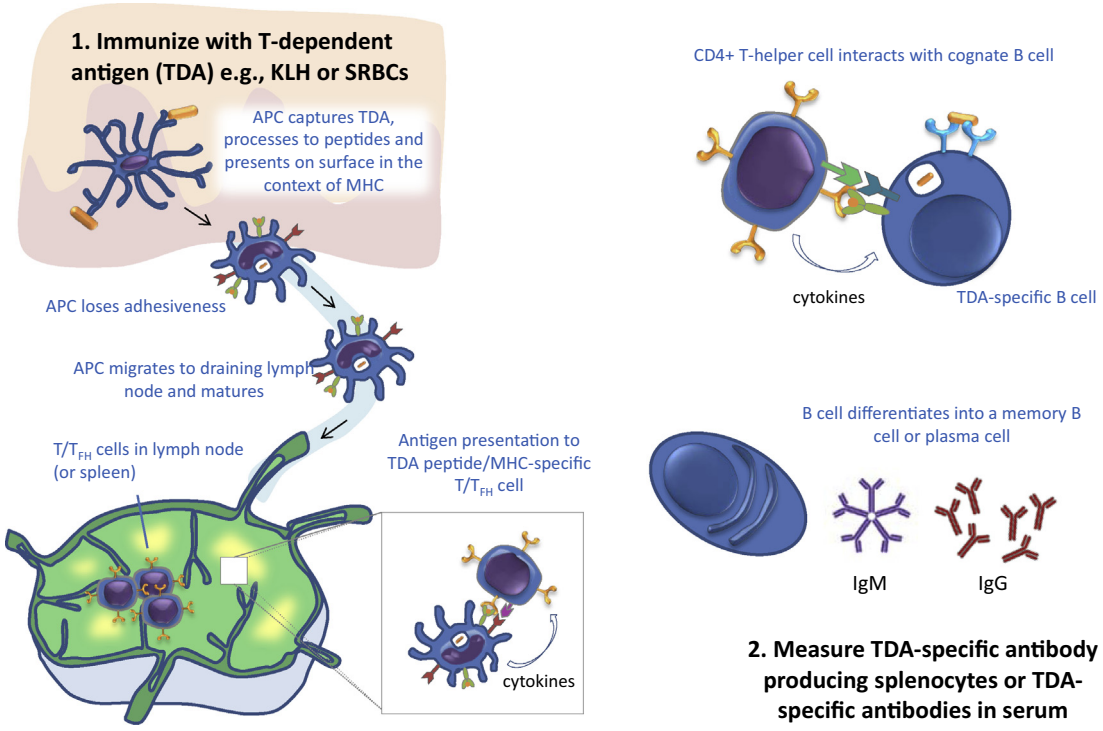

Fig. 1. Schematic representation of the TDAR. APC = antigen presenting cell, TDA = T-dependent antigen, MHC = major histocompatibility complex, T_{FH} = follicular T helper cell.