ELSEVIER

Critical Reviews in Oncology/Hematology 83 (2012) 276–282

CRITICAL REVIEWS IN
Oncology
Hematology
Incorporating Geriatric Oncology

www.elsevier.com/locate/critrevonc

Missing: A diagnostic technique to enumerate antigen-specific T cells

Melinda Shelley Suchard *

Molecular Medicine and Haematology, National Health Laboratory Service, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road Parktown, Johannesburg 2192, South Africa

Accepted 9 November 2011

Contents

Abstract

T lymphocytes are responsible for immune responses against pathogens, immune surveillance against cancer and maintenance of tolerance to self. While techniques available to detect antigen-specific T cells have been well described, there is a missing technique in our repertoire. While fluorescent multimers can be used for limited research applications, there is no existing technique suitable for detection of antigen-specific T cells in a diagnostic setting. The absence of such a technology has inhibited the search for "correlates of protection" against infectious, autoimmune or malignant disease. This critical review of existing methods will highlight the limitations of the data on which our current understanding of the immune system is based, in an effort to stimulate development of improved techniques.

Keywords: Method; Interferon gamma; T cell; T lymphocyte; Immune; Tuberculosis; HIV; Cancer; Tetramer; HLA

1. Introduction

T lymphocytes, together with antibody responses, are responsible for immune responses against pathogens. Also, T lymphocytes maintain tolerance to self antigens and aberrant responses may result in autoimmune disease or malignancy. Much has been written about techniques available to detect antigen-specific T lymphocytes (T cells). What is not usually mentioned is the *absence* of any technique applicable to the clinical setting. This vacuum immediately affects every clinician or scientist researching infectious, autoimmune or malignant disease; but is not often appreciated by non-immunologists. In the current search for biomarkers for malignant and infectious diseases and attempts to develop tumour vaccines, it is vital to draw attention to this missing technology and to stimulate new ideas for its realisation. It is

* Tel.: +27 11 489 8805; fax: +27 86 511 7773.
E-mail addresses: Melinda.suchard@nhls.ac.za, thesuchards@telkomsa.net

1040-8428/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.critrevonc.2011.11.002

also important to highlight the paucity of data on which our current understanding of T cell mediated conditions is based.

2. Antigen-specific T lymphocytes

Each T lymphocyte has a T cell receptor with a unique structure as a result of V(D)J recombination. This unique structure allows each T cell to respond to a unique peptide fragment presented via a MHC (Major Histocompatibility Complex, in humans known as HLA for Human Leucocyte Antigen) molecule [1–3]. Following stimulation of a naïve T cell, the T cell undergoes proliferation to multiple daughter cells and differentiation to become an effector or memory T cell clone. This process is analogous to a naïve B cell becoming stimulated via its B cell receptor and differentiating into a clone of high-affinity, class-switched (IgG or IgA-producing) plasma cells or memory B cells.

3. Antigen-specific antibodies

Measurement of antibody classes (IgM, IgG, IgA) against particular antigens has become the mainstay of diagnosis for many clinical conditions. The presence of IgG, rather than IgM, to a particular infectious agent is taken as a sign of prior exposure of B lymphocytes to the organism or antigen, and the antibody titre often reflects the time course of the infection. Examples include presence of IgG to Hepatitis B core antigen as a marker of prior natural Hepatitis B infection [4,5], or the presence of IgG to Rubella to indicate Rubella immunity in pregnant women [6]. Antibodies may be employed to diagnose autoimmune diseases, such as the presence of anti-acetylcholine antibodies to diagnose myasthenia gravis or the presence of anti-tissue transglutaminase antibodies to aid diagnosis of coeliac disease [7,8].

4. Detection of antigen-specific T cells in comparison with detection of antigen-specific antibodies

While the presence of specific antibodies has become a routine diagnostic tool, detection of antigen-specific T cells remains limited largely to research applications. Tuberculosis (TB) diagnosis is a notable exception, with use of ELISpot technology (T-SPOT.*TB*, Oxford Immunotec) and T cell cytokine production (QuantiFERON-TB Gold, Cellestis) showing utility in certain settings [9]. The paucity of diagnostic applications for detection of antigen-specific T cells is likely due to technical limitations hampering current detection methodologies.

Detection of antigen-specific antibodies is relatively simple. Enzyme immunoassays involve immobilisation of antigen on a solid support (ELISA well, bead, strip etc.) and addition of patient serum. Antigen-specific antibody in the patient serum binds to the immobilised antigen. Any excess unbound antibody is washed off and then bound antibody is detected by a labelled secondary anti-human antibody in a "sandwich" format. The label may be an enzyme which enacts a colour or luminescence change with addition of substrate. The amount or rate of colour change is proportional to amount of antigen-specific antibody in the patient sample. The same principles, however, do not apply to detection of antigen-specific T cells, as T cells will not bind directly to their cognate (corresponding) peptide antigen even if the antigen is immobilised on a solid support. The T cell receptor binds only to its cognate peptide presented in conjunction with a self-MHC molecule (Fig. 1). To immobilise the antigen on a solid support would entail immobilisation of peptide in conjunction with MHC molecule, and would be applicable only to patients having an identical HLA molecule. As HLA molecules are encoded by the most polymorphic human gene locus known, with approximately 300 HLA-A, 600 HLA-B and 100 HLA-C alleles already identified, [10], any one peptide–MHC complex can bind to T cells from very few individuals. Therefore, this approach is not an attractive diagnostic prospect.

5. Shortfalls of existing methods for detection of antigen-specific T cells

The principle of a known HLA molecule presenting a known peptide forms the basis of a highly informative research technique known as multimer staining. This encompasses tetramers (available from National Institute of Health tetramer facility and Beckman Coulter), Pro5®pentamers (ProImmune), Ultimers™ (ProImmune) and MHC Dextramers™ (Immudex)). Multimers are fluorescently tagged MHC–peptide complexes that allow flow cytometric detection of T cells harbouring a cognate T cell receptor [11,12]. The MHC–peptide complex is multimerised to increase binding avidity for the T cell receptor and increase duration of the multimer-T-cell-receptor binding [13]. Multimer staining can be optimised to detect antigen specific T cells at very low frequencies [14–16]. Multimer studies have shown that frequencies of antigen specific $CD8^+$ T cells are decreased during active TB infection in comparison with latent infection and return to normal after treatment [17]. They have also shown numbers of cytomegalovirus specific T cells post-haemopoeitic stem cell transplant can be used to monitor risk of CMV disease [18]. Tetramer studies have also shown differences in antigen-specific T cells in allergic compared with non-allergic individuals [19]. These studies illustrate the importance of monitoring frequencies of antigen specific T cells in infectious and immune-mediated disease. These studies have however, been limited to patients of selected HLA types.

It is possible to develop an array of MHC–peptide complexes from one or multiple MHC types [20–22]. The cost of manufacture of these multimers in an array format, however, is currently prohibitive. Also, as peptide fragments are