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EASL meeting report

Immunological techniques in viral hepatitis

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The need to quantitate and monitor immune responses of large patient cohorts with standardized techniques is increasing due to the growing range of treatment options for hepatitis B and hepatitis C, the development of combination therapies, and candidate experimental vaccines for HCV. In addition, advances in immunological techniques have provided new tools for detailed phenotypic and functional analysis of cellular immune responses. At present, there is substantial variation in laboratory protocols, reagents, controls and analysis and presentation of results. Standardization of immunological assays would therefore allow better comparison of results amongst individual laboratories and patient cohorts. The EASL-sponsored and AASLD-endorsed Monothematic Conference on Clinical Immunology in Viral Hepatitis was held at the University College London, United Kingdom, Oct 7–8, 2006 to bring together investigators with research experience in clinical immunology of hepatitis B virus (HBV) and hepatitis C virus (HCV) infections for in-depth discussion, critical evaluation and standardization of immunological assays. This report summarizes the information presented and discussed at the conference, but is not intended to represent a consensus statement. Our aim is to highlight topics and issues that were supported by general agreement and those that were controversial, as well as to provide suggestions for future work.

***Keywords*: Hepatitis B virus; Hepatitis C virus; T cell; B cell; Lymphocyte; Dendritic cell; Antibody; Proliferation; ELISpot; Vaccine**

1. Introduction

In preparation of the conference, speakers and moderators completed a questionnaire on immunological research practices instituted in their laboratories. The presentations at the conference reviewed the advantages, disadvantages and pitfalls of individual techniques according to specific topics (Table 1). The conference participants consisted of senior investigators (59% MDs, 32% PhDs), the majority with hands-on experience in the discussed techniques during the past 5 years in either academic (63%) or industry (18%) settings (Table 2). Eighty-one percent of the conference participants were actively studying immune responses either

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Abbreviations: ACD, acid citrate dextrose; CFSE, carboxyfluorescein diacetate succinimidyl ester; CPDA, Citrate phosphate dextrose; cpm, counts per minute; CTL, cyotoxic T-cell; DC, dendritic cells; DMSO, dimethylsulfoxid; EDTA, ethylenediaminetetraacetic acid; ELISpot, enzyme-linked immunospot; FBS, fetal bovine serum; FCC, flow cytometry cytotoxic T-cell assay; GM-CSF, granulocyte macrophage colony-stimulating factor; HBV, hepatitis B virus; HCV, hepatitis C virus; HCVpp; retroviral HCV pseudoparticle; IC70, 70% inhibitory capacity; mDC, myeloid dendritic cell; MHC, major histocompatibility complex; MIP-1α, macrophage inhibitory protein 1α; MIP-1β, macrophage inhibitory protein 1β; NK, natural killer cell; NKT, natural killer T-cell; PBMC, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; pDC, plasmacytoid dendritic cell; RANTES, regulated upon activation, normal T-cell expressed, and secreted; RPMI, Roswell Park Memorial Institute cell culture medium; TLR, toll-like receptor; TNF-α, tumor necrosis factor α.

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Table 1
Faculty participants

Faculty member	Institution	Topic
Barbara Rehermann	National Institutes of Health, USA	Immunological Assays in Hepatitis: The Problem of Standardization
Session on Innate Immune Responses (Moderator: David Adams)		
Pablo Sarobe	University of Navarra, Spain	Dendritic Cells
Ulrich Spengler	University of Bonn, Germany	NK Cell Assays
Ex vivo Quantitation of T Cell Responses (Moderator: Nikolai Naoumov)		
Heiner Wedemeyer	Hannover Medical School, Germany	ELISpot Assays
Paul Klenerman	University of Oxford, UK	HLA class I and II Tetramers and Intracellular Cytokine Staining
Michelina Nascimbeni	Institute Cochin, France	FACS-based Assays for Cytokine Production
Ian McInnes	University of Glasgow, UK	Lessons from Analysing T Cell Responses in Rheumatology
Characterizing T Cell Specificity (Moderator: Barbara Rehermann)		
Georg Lauer	Massachusetts General Hospital, USA	T Cell Epitope Mapping
Gabriele Missale	University of Parma, Italy	Generation and Application of T Cell Lines and Clones
David Bowen	Columbus Children's Research Institute, USA	Analysis of Liver-Infiltrating Lymphocytes
Specific CD4, CD8 and B Cell Effector Functions (Moderator: Vincenzo Barnaba)		
Helmut Diepolder	University of Munich, Germany	Assays for CD4 T Cell Functions
Kyong-Mi Chang	University of Pennsylvania & VA Medical Center, USA	Assays for Cytolytic Effector Functions
Mario Mondelli	University of Pavia, Italy	Characterization of B Cells
The Virus and Modeling of Virus Host Interaction (Moderator: Jean-Michel Pawlotsky)		
Jane McKeating	University of Birmingham, UK	Analysis and Quantitation of Neutralizing Antibodies
Stuart Ray	Johns Hopkins University, USA	Analysis of Viral Sequence Evolution in Relation to Immune Selection Pressure
Alan Perelson	Los Alamos National Laboratory, USA	Mathematical Modeling

during the natural course of viral hepatitis (35%), during therapy with interferon/ribavirin and/or antivirals (25%) or during vaccine and/or immunotherapy trials (21%). Two-thirds of the audience had coauthored publications on immune responses in viral hepatitis and/or other diseases (Table 2). During the conference, the audience was polled on the same questions on laboratory practices as the speakers, which served as a starting point for discussions.

2. History of immunological techniques employed to study immune responses in viral hepatitis

Historically, the first assays to study HBV- and HCV-specific T-cell responses were based on in vitro expansion of virus-specific T-cells. Proliferation assays were the first approach to assess CD4 T-cell responses to HBV antigens. Peripheral blood mononuclear cells (PBMCs) were stimulated with recombinant viral proteins. CD4+ T-cell lines and clones were established from blood or liver of infected patients [1–4] and chimpanzees [5,6]. For assessment of CD8+ T-cell responses, PBMCs were stimulated with short synthetic peptides for 2–3 weeks and then assessed for their ability to kill patient-derived autologous EBV-B cell lines loaded with the same peptide or infected with recombinant vaccinia viruses [7–9]. The advantage of this technique was its high sensitivity, because it combined the in vitro expansion of low-frequency HBV-specific CD8 T-cell populations with an assessment of their effector function. Disadvantages were the preselection of peptides based on HLA-binding motifs and the possible loss of low-avidity T-cells during in vitro expansion.

While in vitro expansion techniques were very useful for the identification of CD4+ and CD8+ T-cell epitopes, it soon became necessary to quantitate the number of epitope-specific T-cells. Using an in vitro expansion technique, this was first performed with limiting dilution cultures to estimate the frequency of cytotoxic T-cell precursors [10,11]. When ex vivo techniques such as ELISpot and tetramer-technology became available, however, it became evident that the number of virus-specific T-cells in the blood was much higher than previously estimated [12]. Both the ELISpot and tetramer assays allowed direct ex vivo quantitation of virus-specific T-cells without in vitro expansion. In addition, the tetramer technology allowed the detection of virus-specific T-cells independent of their function. Further developments concerned the synthesis and use of large panels of overlapping peptides spanning entire viral proteins. This facilitated a comprehensive assessment of all T-cell specificities, quantitation of both CD4+ and CD8+ T-cell responses in a single assay in the context of all given HLA-alleles and, when peptide pools were set up in a matrix format, simultaneous identification of candidate epitopes [13,14].