

Cellular Immunity and CMV

with

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Why so much interest in cytomegalovirus immunology?

Giuseppina Li Pira & Fabrizio Manca: Cytomegalovirus (CMV) is a paradigm for host-pathogen interactions. The containment of CMV infection in a latent state after primary infection (as for other herpesviruses) depends on effective cellular immunity mediated by CD8 and CD4 lymphocytes, for which there is experimen-

tal and clinical evidence. The mouse model demonstrates that different cell populations cooperate to control CMV infection (1). In humans, AIDS patients and recipients of organ grafts or hemopoietic stem cell (HSC) grafts, have a defective cellular immunity and are prone to CMV reactivation leading to severe complications (2).

How knowledge in cellular and viral immunology can benefit these patients?

Giuseppina Li Pira & Fabrizio Manca: Progresses have been made recently to understand CD4 and CD8 responses to pathogens (and to CMV in particular). Immune responses have been dissected at the level of immunodominant epitopes, mechanisms of antigen uptake, processing and presentation, T cell activation and effector functions, clonal make-up, identification and enumeration of specific T cells. Several reviews on these topics have been recently collated (3). These studies facilitate diagnosis of cellular immunity and permit adoptive reconstitution of immunocompromised patients with the specific T cells they need.

With respect to diagnostics, it has been hard to dissect the functions of T lymphocytes, even though cellular immunity is sustained by about 1 kg of such cells. In fact, in the early days intradermal tests could probe cellular immunity, but little was known about the significance

of the macrophage-mononuclear infiltrate that accounts for DTH. Novel assays have been developed in the last two decades. The assays currently available for specific T cells can be either static or dynamic, as we described in a recent review (4). Static assays are based on flow cytometric identification of antigen specific T cells that have bound a specific reagent (e.g. a peptide-MHC tetramer). The tetramer assay is straightforward, but depends on the HLA type of the subject and on previous definition of immunodominant epitopes. Conversely, the functional assays identify antigen responsive cells by indirect criteria, such as cytokine production or proliferation. We can predict that under the strong pressure for better assays and thanks to our improved knowledge of lymphocyte functions, the current methods for cellular immunity will become standard routine and more assays will be developed.

So what can we do for patients with defective CMV immunity?

Giuseppina Li Pira & Fabrizio Manca: Immunology must proceed hand in hand with virology at the diagnostic level, when the risk for a patient has to be defined and

when therapeutic options must be proposed. Virological assays showing active viral replication, in combination with defects of CMV specific cellular immunity, define

Can you summarize the state of the art in this field?

Giuseppina Li Pira & Fabrizio Manca: In their seminal studies, Riddell and Greenberg (5) generated CMV specific CD8 CTL clones from the HSC donors and reinfused them into the patient to prevent or treat CMV pneumonia. The trials were successful, but the complexity of the method to produce CTL clones based on CMV infected fibroblasts prevented extended use. Cellular immunologists were fascinated by this perspective (5) and engaged in studies to define epitopes (6), optimal antigen presenting cells (7), methods for positive selection of specific cells (8). Several reports demonstrated that APC can be constructed to express CMV epitopes and that new antigenic prepara-

the high risk patient. Therefore effective antiviral drugs can be used to curb viral replication. Nevertheless the drugs are toxic for bone marrow cells, which may delay immunoreconstitution of the specific lymphocytes and drug resistant CMV strains may appear. Thus adoptive cellular therapy can be entertained.

tions can replace CMV infected fibroblasts (7). Nevertheless, since therapeutic cells must be prepared with simple procedures, not all of these studies are clinically applicable. Three groups recently contributed with therapeutic trials. Einsele et al (9) and Peggs et al (10) generated and expanded mixtures of CD4 and CD8 cells specific for CMV from HSC allodonor. Reinfused cells did not cause GVHD, since alloreactive contamination was undetectable, but resulted in clinical benefit. Cobbold et al (11) used tetramers to select CMV specific CD8 cells, that were reinfused without *in vitro* expansion. Also in this case the clinical benefit and the *in vivo* persistence were shown.

Which progresses do you envisage in the future?

Giuseppina Li Pira & Fabrizio Manca: The culture systems should be simpler and more efficient, so the treatment can be made available to more patients, without being an experimental therapy. Simpler means fewer steps for cell separation, selection and expansion. Use of sealed systems may increase safety. Selected institutions may provide a contract service, so to avoid expensive replication of GMP facilities. Increased efficiency means use of better antigen presenting cells, such as dendritic cells, or powerful antigenic formulations like cocktails of synthetic peptides (12,13). These concepts apply to CD8 and to CD4 cell lines. We also envisage in the future production of simplified kits for the generation of T cell lines and introduction of antigenic peptide libraries derived from other opportunistic pathogens.

We are confident that the use of peptides as antigens, cytokines for T cell expansion or dendritic cell differentiation-maturation and improved bioreactor-type culture vessels will make adoptive therapy with specific lymphocytes available to immunocompromised patients as a standard treatment.

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