MINI-REVIEW

Immunopathology caused by impaired CD8⁺ T-cell responses

Peter Aichele¹,², Christoph Neumann-Haefelin³, Stephan Ehl¹,², Robert Thimme³, Toni Cathomen²,⁴, Melanie Boerries⁵,⁶ and Maike Hofmann³

¹ Institute for Immunodeficiency, Faculty of Medicine, Medical Center—University of Freiburg, Freiburg, Germany
² Center for Chronic Immunodeficiency (CCI), Faculty of Medicine, Medical Center—University of Freiburg, Freiburg, Germany
³ Department of Medicine II (Gastroenterology, Hepatology, Endocrinology and Infectious Diseases), Faculty of Medicine, Medical Center—University of Freiburg, Freiburg, Germany
⁴ Institute for Transfusion Medicine and Gene Therapy, Medical Center—University of Freiburg, Freiburg, Germany
⁵ Institute of Medical Bioinformatics and Systems Medicine, Faculty of Medicine, Medical Center—University of Freiburg, Freiburg, Germany
⁶ German Cancer Consortium (DKTK), Partner Site Freiburg, and German Cancer Research Center (DKFZ), Heidelberg, Germany

Recent findings indicate that many immunopathologies are at their roots a consequence of impaired immune responses (“too little” immunity) and not the result of primarily exaggerated immune responses (“too much” immunity). We have summarized this conceptional view as “IMPATH paradox.” In this review, we will focus on impaired immune reactions in the context of CD8⁺ T-cell-mediated immunopathologies. In particular, we will exemplify this concept in two disease models: Virus-triggered primary hemophagocytic lymphohistiocytosis, an inflammatory syndrome caused by genetically impaired cytolytic functions of T cells, and viral hepatitis, where T-cell exhaustion is a major underlying mechanism for impaired effector functions. In both situations, T cells fail to eliminate the source of immune stimulation, which usually serves as an important negative feedback loop curtailing immune reactions. Persistent antigen presentation by APCs and/or infected cells results in continuous stimulation causing chronic inflammation and immunopathology mediated by residual T-cell functions. Hence, immune stimulation or reconstitution rather than immune suppression may be strategies for therapeutic interventions.

Keywords: CD8⁺ T cells · chronic viral hepatitis · immunopathology · primary hemophagocytic lymphohistiocytosis · T-cell exhaustion

See accompanying Commentary by Ehl and Thimme.
Figure 1. Immunoopathology in primary HLH and chronic viral hepatitis. Impaired CD8⁺ T-cell responses fail to eliminate stimulatory signals, particularly, APCs in primary HLH and virus-infected hepatocytes in chronic viral hepatitis, resulting in ongoing CD8⁺ T-cell stimulation. Consequently, a persisting inflammatory response is established comprising cytokines, recruitment and activation of other immune cells that subsequently lead to immunopathology. Created with BioRender.com.

CD8⁺ T-cell effector functions: A double-edged sword

CD8⁺ T cells acquire cytotoxic effector function upon activation by APCs, subsequent clonal expansion and differentiation [1, 2]. Activated CD8⁺ T cells egress from the secondary lymphoid organs to patrol peripheral tissues and eliminate infected or malignant cells. CD8⁺ T cells induce apoptosis of target cells by FasL and by polarized exocytosis of cytotoxic granules containing the pore-forming molecule perforin and serine proteases like granzyme B [3]. To exert their cytotoxic function, CD8⁺ T cells need to establish direct cell-to-cell contact in a T-cell receptor/peptide-loaded MHC class I molecule-dependent manner and the formation of an immunological synapse [4]. In addition, CD8⁺ T cells secrete proinflammatory, antiviral, and antiproliferative cytokines like IFN-γ and TNF as well as chemokines. Hence, CD8⁺ T cells are well-equipped to efficiently eradicate harmful cells. However, these same functions can also render CD8⁺ T cells pathogenic. To restrict their pathogenic potential, CD8⁺ T-cell effector functions are kept in check at different levels, for example, activation, acquisition of the cytotoxic potential, formation of the immunological synapse, release of the cytotoxic granules, and shutdown of effector functions. As soon as this tight regulation of effector functions is out of balance, CD8⁺ T cells can mediate immunopathology. In the classical view, immunopathology is associated with an overshooting immune response, inducing severe tissue destruction, as it is well-established in mouse models of lymphocytic choriomeningitis virus (LCMV) infection. Dependent on inoculum dose, route of infection and virulence of the LCMV isolates, a virus-triggered immunopathology is observed. An example is LCMV-triggered lethal choriomeningitis after intracerebral infection of WT mice [5, 6]. Interestingly, perforin-deficient mice failed to develop this disease although the brain was infiltrated by CD8⁺ T cells, consistent with the prediction of conventional models of immune-mediated diseases that impaired effector functions attenuate immunopathology [7]. However, not only overshooting but also impaired CD8⁺ T-cell effector functions can result in immunopathology. In this review, we will discuss the paradoxical concept of “immunopathology caused by impaired CD8⁺ T-cell responses” on the basis of two diseases: hemophagocytic lymphohistiocytosis (HLH) and chronic viral hepatitis. In both disorders, functionally impaired CD8⁺ T cells fail to eliminate stimulatory signals, resulting in persisting inflammatory responses and, thus, chronic immunopathology (Fig. 1).

HLH: Impaired T-cell cytotoxicity by genetic defects

Primary HLH is a life-threatening syndrome of immune dysregulation characterized by systemic hyperinflammation and cellular immunopathology in various tissues due to infiltration of uncontrolled activated T cells and macrophages, accompanied by massive cytokine secretion (IFN-γ, TNF, IL-6, IL-18) [8–10]. Because of this loss in immune homeostasis, HLH patients present with prolonged fever, hepatosplenomegaly, cytopenia, hemophagocytosis, liver disease, and neurological manifestations. A pathogenic trigger is often not identified; however, viral infections (EBV, CMV) can be potent disease inducers [11]. Primary HLH is usually a disease of early childhood and without treatment, the outcome is lethal. Untreated patients have a survival prognosis of about 2 months. First-line therapeutic interventions focus on immunochemotherapy based on aggressive immunosuppression and/or targeting cytokines/cytokine receptor signalling pathways to resolve hyperinflammation, eliminate activated immune
cells, and dampen the cytokine storm, with the aim to stabilize the patient for allogeneic HSC transplantation, the only curative treatment so far [12–16]. Familial forms of HLH (FHL) are caused by genetic defects affecting lymphocyte cytotoxicity [17]. Mutated genes encode for proteins that are involved in the biogenesis, intracellular transport, release and function of perforin-containing cytoplasmic granules of T- and NK cells. This includes defects in PERFORIN (FHL-2), UNC13D (FHL-3), SYNTAXIN-11 (FHL-4), and MUNC18-2 (FHL-5), all associated with impaired cytotoxicity [18]. But why does impaired lymphocyte cytotoxicity lead to uncontrolled immune responses, hyperinflammation, and severe immunopathology? Preclinical animal models were of great value for the current understanding of HLH pathogenesis [19–23]. After infection with LCMV as trigger, perforin-deficient mice recapitulate the clinical picture of FHL, as seen in patients [20–22]. Typically, LCMV infection induces a strong CD8+ T-cell response in WT mice, which eliminates the virus within 8–10 days. In contrast, in perforin-deficient mice, the control of LCMV infection is lost and virus persists in all organs due to the impaired cytotoxicity of activated T cells [24, 25]. This results in prolonged and excessive antigen presentation by virus-infected cells and activated APCs, like DCs [26]. Hence, lymphocytes are stimulated continuously, promoting T-cell expansion, excessive cytokine production (e.g., “IFN-γ storm”), macrophage hyperactivation causing further cytokine release, tissue infiltrations, and severe immunopathologies. As a consequence of this vicious cycle of persisting inflammatory response without antigen elimination, LCMV-triggered perforin-deficient mice succumb to lethal HLH, driven by activated dysfunctional CD8+ T cells and IFN-γ leading to chronic immunopathology and finally multiorgan failure [20, 21, 27]. Thus, impaired cytotoxicity of CD8+ T cells results in the failure to terminate immune responses since the negative feedback loop eliminating the stimulating DCs is missing. LCMV infection itself does not cause tissue damage as the virus is noncytopathic, supporting the view that HLH in perforin-deficient mice represents a virus-triggered immunopathology. Accordingly, the depletion of the dysfunctional CD8+ T cells prevents LCMV-triggered HLH in perforin-deficient mice. Treated mice exhibited no signs of disease and survived for a long term [21, 22]. In summary, virus-triggered primary HLH is an example of a life-threatening immunopathology in the context of impaired CD8+ T-cell cytotoxic functions.

Chronic viral hepatitis: Impaired T-cell effector functions by virus/host adaptation

Chronic viral hepatitis is a major global health burden, affecting an estimate of 350 million people. The main causative agents are hepatitis B virus (HBV) and HCV. Infections with HBV and HCV cause necroinflammatory liver disease that can progress to liver cirrhosis and ultimately to end-stage liver disease or liver cancer. Since the approval of direct-acting antivirals, chronic HCV infection can be cured [28]. In contrast, treatment of chronic HBV infection with nucleos(t)ide analogs (NUC), the current standard of care, represses viral replication but does not lead to viral clearance [29, 30]. Both viruses are hardly cytopathic and the associated pathomechanism of hepatitis represents virus-triggered immunopathology [31]. Virus-specific CD8+ T cells are major determinants of the outcome and pathogenesis. Indeed, strong virus-specific T-cell responses are detectable in self-limiting viral hepatitis but chronic viral hepatitis is characterized by impaired virus-specific CD8+ T-cell responses [32]. Therefore, reconstituting CD8+ T-cell functions is considered a promising target for novel immunotherapeutic interventions aiming at cure of the immunopathology associated with chronic HBV infection [29, 30].

The impaired virus-specific CD8+ T-cell response is reflected by the low frequencies of circulating HBV- and HCV-specific CD8+ T cells in chronically infected patients. Importantly, these impaired virus-specific CD8+ T-cell responses are not “nonfunctional” but rather dysfunctional and retain sufficient activity to mediate chronic immunopathology and, thus, significantly contribute to ongoing liver disease [33]. Two partially overlapping mechanisms have so far been identified as underlying virus-specific CD8+ T-cell impairment in chronic viral hepatitis: viral escape and T-cell exhaustion [34]. On the one hand, in viral escape, sequence mutations in CD8+ T-cell epitopes of the viral genomes result in reduced recognition of infected hepatocytes by CD8+ T cells [35]. Yet, recognition is rarely completely abrogated and, therefore, virus-specific CD8+ T-cell effector activity, even though reduced, is persistently elicited, contributing to ongoing pathology. CD8+ T-cell exhaustion, on the other hand, represents a physiological adaptation of virus-specific CD8+ T cells in chronic viral infection to limit direct CD8+ T-cell-mediated immunopathology. However, this is achieved at the expense of viral control [36]. Exhausted virus-specific CD8+ T cells are characterized by altered effector functions, mainly reduced cytotoxicity and IFN-γ, TNF, and IL-2 cytokine secretion but increased production of chemokines like XCL-1 and CCL3/4 compared to effector CD8+ T cells arising in self-limiting viral infections [36, 37]. With this functional profile, exhausted CD8+ T cells exhibit an impaired capacity to directly combat virus-infected cells but fuel the nonspecific inflammatory response by recruiting other immune cells. Consequently, a vicious cycle of a persisting inflammatory and residual cytotoxic response without viral elimination is generated, resulting in chronic immunopathology.

Functional impairment of exhausted CD8+ T cells is linked to a distinct epigenetic, transcriptional, metabolic, and phenotypic profile indicative of a discrete T-cell differentiation program [36]. The epigenetic and transcriptomic signatures of exhausted virus-specific CD8+ T cells are retained like a molecular scar in virus-specific CD8+ T cells even after clearance of chronic viral infection [38–41]. Thus, dysfunction is imprinted and a functionally impaired, memory-like CD8+ T-cell response is maintained [42]. The thymocyte selection-associated high mobility group box protein, TOX, has recently been identified as a master regulator linking the transcriptional and epigenetic program of CD8+ T-cell exhaustion [43–45]. TOX seems to act as a rheostat for the
Conclusions for future therapeutic strategies

Based on the concept that immunopathology may be paradoxically caused by impaired immunity (“IMPATH paradox”), it is intriguing to propose that strengthening of the T-cell response by immune stimulation or reconstitution will lead to an abrogation of the underlying vicious cycle of immunopathology. The goal is to improve T-cell functions, allowing them to terminate chronic stimulation, for example, mediated via APCs in HLH or infected hepatocytes in chronic viral hepatitis. Accordingly, a combination of antivirals and boosting immunotherapy is considered to be likely required for HBV cure [29, 30]. In particular, several experimental immunotherapeutic approaches aim at strengthening the CD8+ T-cell response in chronic HBV infection. These attempts comprise therapeutic vaccination; checkpoint blockade in combination with costimulatory or adjuvant molecules; and adoptive therapy with genetically modified, autologous T cells (e.g., with redirected HBV-specific TCRs or chimeric antigen receptors) [29, 30]. With respect to downregulation of hyperinflammation as first-line therapy in virus-triggered HLH, a paradigm shift from aggressive immunosuppression to immune reconstitution may be the key for future successful therapeutic concepts. Genetic engineering to correct autologous hematopoietic stem cells is a key strategy in achieving this goal, in particular, if combined with newborn screening to identify patients before immune activation. However, also in active HLH, partial reconstitution of cytolytic activity by transfer of functional virus-specific T cells (adoptive T-cell therapy) may be a promising therapeutic strategy to ameliorate active HLH by eliminating the virus trigger, thereby terminating the continuous stimulation. As a proof of principle, previous work shows that prophylactic transfer of polyclonal WT or gene-corrected CD8+ T cells into HLH-prone mice before the triggering of disease protects mice from acute HLH [49, 50]. Although at first glance, counterintuitive, therapeutic intervention strategies based on adoptive T-cell therapy to enhance immunity under conditions of hyperinflammation during acute HLH are tested currently in preclinical mouse models. If such therapeutic approaches are successful to intervene in active HLH without fueling the hyperinflammation, this may pave the way for a more general rethinking of therapeutic intervention strategies in situations of immunopathology triggered by impaired immune responses.

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Abbreviations: FHL: familial forms of HLH · HBV: hepatitis B virus · HLH: hemophagocytic lymphohistiocytosis · LCMV: lymphocytic choriomeningitis virus

Full correspondence: Maike Hofmann, Department of Medicine II (Gastroenterology, Hepatology, Endocrinology and Infectious Diseases), Medical Center—University of Freiburg, Hugstetter Str.55, 79106 Freiburg, Germany. e-mail: maike.hofmann@uniklinik-freiburg.de

Peter Aichele, Institute for Immunodeficiency, Medical Center—University of Freiburg, Hermann-Herder Str. 11, 79104 Freiburg, Germany. e-mail: peter.aichele@uniklinik-freiburg.de

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