Harnessing HLA-E-restricted CD8 T lymphocytes for adoptive cell therapy of patients with severe COVID-19

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is spreading worldwide and is a now a pandemic virus that has infected almost 5 million individuals, causing 300 000 deaths as of mid-May 2020. Because SARS-CoV-2 is a new virus in humans there are currently no vaccines, monoclonal antibodies or even effective drugs available. Human convalescent plasma transfusion is an option for either prophylactic or therapeutic treatment of patients with coronavirus disease 2019 (COVID-19), but its administration to patients who are affected by severe pulmonary disease is associated with increased risk of transfusion-related acute lung injury. Antibody-dependent enhancement of infection is an additional risk that has already been reported to occur in several viral diseases and involves an enhancement of disease in the presence of certain antibodies. Finally, to date there are relatively few people who have recovered from COVID-19 and who can donate immunoglobulin-containing plasma.[1]

Cluster of differentiation 8 (CD8) cytotoxic T lymphocytes are essential for immune protection against viruses and several studies have shown that high levels of virus-specific CD8 T cells are associated with decrease in virus load and favourable outcome. Interestingly, using human leucocyte antigen (HLA) class I predicted peptide 'megapools', SARS-CoV-2-specific CD8 T cells were identified in the circulation of approximately 70% of COVID-19 convalescent patients.[2]

In a correspondence letter to the *British Journal of Haematology* published on-line on 5 May 2020, Hanley *et al.*3 propose to utilise SARS-CoV-2-specific and HLA-matched cytotoxic T cells prepared from convalescent COVID-19 patients for 'the need of the hour' therapy of patients with COVID-19.

Adoptive transfer of donor-derived and virus-specific CD8 T cells has shown efficacy for the treatment of several different viral infections in immunocompromised individuals.[4]

However, there are problems with adoptive T-cell therapy. First, due to genetic (HLA class I) restriction, it is not possible to utilise allogeneic T cells from unrelated individuals and only donor-derived T cells can be used. Second, donor T cells are expanded *in vitro* by prolonged stimulation with specific virus antigen to achieve sufficient numbers of effector T cells to be infused. This is a major obstacle in situations where CD8 T cells are exhausted and fail to proliferate and expand, as occurs in patients with COVID-19, where peripheral blood CD8 T cells counts are profoundly reduced and CD8 T cells are functionally exhausted.[5] Third, an important side-effect of T-cell therapy is the so-called 'cytokine storm', a massive inflammatory response that contributes to acute respiratory distress and multiple organ failure in patients with COVID-19.[6]

The aim of this letter is to discuss the potential for adoptive therapy of patients with severe COVID-19 with HLA-E-restricted unconventional CD8 T cells.

HLA-E is a non-classical HLA class Ib molecule expressed on all nucleated cells. It is considered as non-classical because it is basically monomorphic. There are in fact only two alleles, HLA-E*01:01 and *01:03, which differ by a single amino acid substitution located outside the peptide binding groove, thus suggesting that the two alleles have an identical epitope-binding repertoire.[7] HLA-E typically binds peptides derived from the signal sequence of other HLA class Ia alleles, and these complexes in turn bind to natural killer (NK) group 2 member A (NKG2A)/CD94 and inhibit NK cell functions. However, most recent studies have demonstrated that HLA-E binds peptides derived from different microbes including cytomegalovirus (CMV), human immunodeficiency virus (HIV), *M. tuberculosis*, etc., and presents them to CD8 T cells.[7] The crystal structure of HLA-E bound to CMV, HIV and *M. tuberculosis*-derived peptides has been very recently resolved[8] and may be then exploited to design peptides capable of activating HLA-E-restricted CD8 T cells.

Human HLA-E-restricted CD8 T cells possess cytolytic and microbicidal activities.[9–10] However, these CD8 T cells in addition to the typical Th1 cytokine interferon γ, also produce interleukin 4 (IL-4), IL-5, IL-13, and to a variable extent IL-10 and transforming growth factor β (TGF-β).[9]

Recent studies in non-human primates have shown that vaccination with simian immunodeficiency virus-derived gag protein expressed in rhesus CMV elicits a potent HLA-E-restricted CD8 T cell response, which causes eradication of a subsequent simian immunodeficiency virus challenge.[11]

Most interestingly, in an experimental mouse model of tuberculosis, Qa-1 (the murine orthologue of HLA-E)-restricted CD8 T cells mediate a protective immune response against *M. tuberculosis* by both killing infected cells and the intracellular bacilli, and also limiting the extent of tissue damage.[12]

More than 1000 clinical trials for the treatment of patients with COVID-19 are registered as of mid-May 2020, with only one study from Singapore investigating the efficacy of adoptive cell therapy with SARS-CoV-2-specific T cells in patients with severe COVID-19 (https://clinicaltrials.gov/ct2/show/NCT04351659).

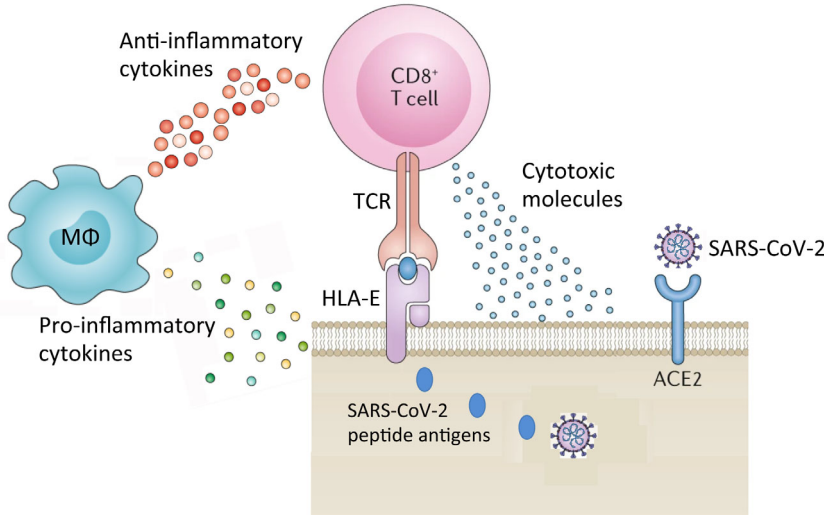

Fig 1. HLA-E molecule in immunity to SARS-CoV-2 infection. HLA-E molecule, expressed on the surface of all nucleated cells, can present SARS-CoV-2-derived peptide antigens to CD8 T cells, which recognise these HLA-E/peptide complexes on the surface of virus-infected cells through their T-cell receptor (TCR). Upon specific recognition, activated CD8 T cells de-granulate and release cytotoxic molecules such as perforin, granzyme and granulysin, which cause killing of infected target cells and of the intracellular virus. In addition, HLA-E-restricted CD8 T cells also produce anti-inflammatory cytokines (IL-4, IL-10, TGF-β), which down-modulate the production of pro-inflammatory cytokines by macrophages and other inflammatory cells, and thus inhibit 'cytokine storm' and reduce the extent of tissue damage.

Here, we would like to propose that the utilisation of HLA-E-restricted CD8 T cells may offer several advantages to improve T-cell immunotherapy in patients with COVID-19, such as the simultaneous capacity to kill infected cells and inhibit intracellular infections, and to reduce the extent of the inflammatory response and limit collateral tissue damage, which is an important component in the pathogenesis of COVID-19 (Fig 1). Another important aspect is represented by the monomorphic model of antigen recognition of HLA-E-restricted CD8 T cells, which permits their utilisation for the global heterogenic population. Moreover, HLA-E-restricted CD8 T cells are unlikely to mount alloreactive responses and cause graft-*versus*-host disease phenomena, making these cells more amenable to 'off-the-shelf' conventional T-cell therapies. In principle, HLA-E-restricted and SARS-CoV-2-specific CD8 T cells could be rapidly and cost-effectively prepared in large numbers from COVID-19 convalescent allogeneic donors, banked and used immediately upon request for patients with severe COVID-19.

Conflict of interest

The authors declare no conflict of interest.

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Unexpected kinetics of anti-SARS-CoV-2 total antibodies in two patients with chronic lymphocytic leukemia

Recently, Baumann *et al.*[1] described the characteristics and outcomes of four patients with chronic lymphocytic leukaemia (CLL) diagnosed with symptomatic COVID-19. The course of the disease was mild, and no patient required admission in an intensive care unit. The authors speculate that the CLL-related immunodeficiency might be beneficial in the outcome of COVID-19 and deserved further investigation. No specific serological testing information was provided. The American Society of Hematology (ASH) has published recommendations on the prevention of COVID-19 in patients with CLL but again not on serological investigations.[2]

However, in the context of COVID-19, serological testing is a valuable strategy for the diagnosis and the characterization of the course of the disease, for identifying convalescent plasma donors, for epidemiological studies as well as for lockdown exit programmes and COVID-19 vaccine development.[3–5] Our group recently reported the validation of an electrochemiluminescence immunoassay (ECLIA) for determination of anti-SARS-CoV-2 total antibodies (Elecsys®, Roche Diagnostics®, Bale, Switzerland) and showed excellent analytical and clinical performance of the assay (95·1% of sensitivity and 100% of specificity), if using an optimized cut-off [i.e. >0·165 cut-off index (COI)].[6] In our cohort of COVID-19 patients, two presented with CLL. Both patients required hospitalization in intensive care units, received hydroxychloroquine for 5 days, and recovered after 18 and 40 days respectively. A control group composed of nine patients nonsuffering from B-cells abnormalities (median age: 79) was included. Among them, seven required hospitalization, all recovered after a mean time of 13·8 days (min-max = 6–24) and five received hydroxychloroquine for 5 days. In this group, total antibodies increased rapidly, were all upper the cut-off by day 14 and remain high overtime; as expected (Fig 1). In comparison, the two patients with CLL showed a different kinetic profile of total antibodies.

The first patient (grey outline) was tested positive for reverse transcription polymerase chain reaction (RT-PCR) 11 days after symptom onset and had COI values of 0·081, 0·100 and 0·167 at day 14, 22 and 24 since symptom onset respectively. The latter COI value was higher than the optimized cut-off (i.e. 0·165 COI) meaning that by using the cut-off of the manufacturer, this patient would not have been considered positive for SARS-CoV-2 antibodies.[6] To confirm our classification for positivity and the kinetics observed, these samples were analyzed on another platform (iFlash1800 from YHLO Biotechnology Co., LTD, Shenzhen, China) for specific SARS-CoV-2 IgG determination. The negative determination on day 14 was confirmed on the iFlash1800 (i.e. 0·67 AU/ml; manufacturer cut-off = 10 AU/ml). At days 22 and 24, samples become positive, with increasing antibody titers (22·65 and 110·53 AU/ml) confirming the late antibody kinetics observed. This patient is known with CLL (Binet group A). The patient presented hypogammaglobulinaemia with serum IgG (6·5 g/l) lower than the age-matched mean [11·5 g/l, reference interval (RI) 7·0–16·0 g/l]. The gamma fraction of the serum electrophoresis also presented low values (values during hospitalization 5·5–7·3 g/l; RI 8·0–13·5 g/l). D-dimer (up to 2·2 μg/ml; RI < 0·5 μg/ml), C-reactive protein (CRP) (up to 117·8 mg/dl; RI < 5 mg/dl), creatinine (up to 1·1 mg/dl; RI 0·3–0·9 mg/dl), lactate dehydrogenase (LDH) (up to 389 U/l; RI < 250 U/l) and white blood cells (WBC) (up to $11{\cdot}3 \times 10^9$/l; RI $4{\cdot}0–10 \times 10^9$/l) were increased, and haemoglobin was found to be low (up to 110 g/l; RI 120–160 g/l). We observed a lymphocyte decrease from $10{\cdot}7 \times 10^9$/l (pre-COVID-19) to $4{\cdot}9 \times 10^9$/l (at admission) (Fig 1). All these features were associated with COVID-19 disease.[7] In the nine control patients, a decrease in lymphocyte count following SARS-CoV-2 infection was also