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Impaired production of TNF-α by dendritic cells of older adults leads to a lower CD8+ T cell response against influenza

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ABSTRACT

Seasonal influenza causes more morbidity and mortality in older adults than in young adults, apparently because of a decline in immune function with increasing age, known as immunosenescence. In this study, we compared the capacity of dendritic cells (DCs) from healthy older adults (≥65 years) with DCs from healthy young adults (20–40 years) to initiate a T cell response against influenza. DCs from older adults were impaired in the induction of influenza-specific CD8+ T cells as compared to DCs from young adults, which was demonstrated by a decreased proliferation, an impaired production of IFN-γ and a reduced expression of the degranulation marker CD107a by CD8+ T cells. Importantly, DCs from older adults produced significantly less TNF-α, showed a decreased expression of HLA class I and had a lower maturation state after influenza virus infection. Supplementing TNF-α increased the expression of HLA class I and of maturation markers and enhanced the induction of the influenza-specific CD8+ T cell response. Together, these findings indicate that the impaired influenza-specific CD8+ T cell response in older adults is associated with a reduced production of TNF-α and with a lower DC maturation. We suggest that the production of TNF-α is a determining factor in the DC-mediated CD8+ T cell response against influenza.

1. Introduction

Seasonal influenza infects people of all ages. In older adults, it causes high morbidity and mortality [1–3], which has been attributed to a deterioration of the immune system with advancing age, known as immunosenescence [4,5]. In older adults, cellular immunity is a major determinant of protection against influenza, in addition to humoral responses [6–8]. Specifically, the antiviral capacity of CD8+ T cells has been associated with protection against influenza in older adults [9]. However, immunosenescence affects the T cell function, e.g. naive T cells are impaired by thymic involution [10]. Similarly, the immune response of antigen experienced cells is affected through an accumulation of highly differentiated effector T cells having a restricted T cell receptor repertoire [10,11], an impaired ability to migrate and a decreased ability to be stimulated by antigen presenting cells [12–14].

T cells are critically dependent on professional antigen presenting cells, including dendritic cells (DCs), for the induction of an effective immune response [15,16]. Immature conventional DCs scavenge their surroundings for antigens. Upon capture of antigen, they migrate to the regional lymph nodes and differentiate into mature DCs [17]. These mature DCs present viral epitopes, increase expression of cell surface markers such as CD80, CD83, CD86 and CD40 and secrete cytokines that are important for the induction of T and B cell responses [16,18]. The efficacy of DCs in inducing T cell responses may vary greatly. DCs that have not matured completely or that produce an altered cytokine profile may be impaired in the induction of influenza-specific T cell responses [19,20]. In general, age-related changes in DC function include a reduced production of cytokines, defective phagocytosis, and an impaired migration of DCs [21–23]. However, other reports showed conflicting results, e.g. for the secretion of TNF-α and IL-6, which are possibly related to differences in the types of cells used or the study design [21,24–26]. Consequently, aging may affect the efficacy of DCs in inducing T cell responses, although it is not clear how or whether it is related to specific DC subsets.

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Table 1
Study population.

Group	Complete study population		HLA-A2+ population	
	No. subjects	Mean age (±SD)	No. subjects	Mean age (±SD)
Young	44	33 (±4.8)	14	34 (±5.6)
Old	51	74 (±4.4)	15	73 (±5.9)

Several types of DCs have been described. Under steady state conditions, conventional DCs as well as other relevant types, e.g. plasmacytoid DCs (pDCs) are present in the lymphoid tissues and organs [27,28]. pDCs respond to infection or to an inflammatory stimulus by a massive production of type I interferons and by presenting antigen to T cells [29]. In older adults, pDCs are impaired in inducing a CD8+ T cell response against influenza [30].

Under inflammatory conditions, monocytes that are recruited to the inflammatory focus develop into so-called inflammatory DCs [27,31]. The generation of these inflammatory DCs is dependent on GM-CSF [28]. They play an important role in the induction and regulation of immune responses, such as by processing and presenting antigen to T cells, and by producing pro-inflammatory mediators including TNF-α [18,27,32–34]. A specific type of inflammatory DCs (Tip DCs) produces iNOS and TNF-α and was found essential for the proliferation of influenza-specific CD8+ T cells in the infected lung [35]. The development of Tip DCs may be modeled in vitro by culturing monocytes with GM-CSF and interleukin-4 (IL-4) to produce monocyte-derived DCs (moDCs) [28]. Several characteristics of how aging affects the function of moDCs have been described, showing contrasting results which may related to differences in the experimental models used [25]. However the effect of aging on the competence of inflammatory DCs to activate CD8+ T cells, which are a major component of the protective response in older adults against influenza, has not been described.

In this study, we evaluated the capability of moDCs from young and older subjects to induce a CD8+ T cell response against the HLA-A2-restricted epitope of the matrix protein (GILGFVFTL; $M1_{58–66}$). This is the main target for the influenza-specific CTL response in HLA-A2+ individuals [36,37]. We further employed the model to identify essential differences in the induction of T cell responses by DCs from older and young individuals. Our data demonstrate that DCs from older adults have an impaired maturation, a decreased production of TNF-α and a reduced capacity to mount a CD8+ T cell response against seasonal influenza. More importantly, the impaired DC function could be restored by supplementing TNF-α; this indicates that impaired production of TNF-α by DCs plays a critical role in mounting an effective immune response against seasonal influenza.

2. Materials and methods

2.1. Ethics statement

This study was approved by the Medical Ethical Committee of the Utrecht University Medical Center and was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. All participants provided written informed consent after the nature and possible consequences of the studies had been fully explained.

2.2. Study participants

In the summer of 2008, 44 young healthy adults (20–40 years; 13 male) and 51 healthy older adults (≥65 years; 27 male) were recruited in the Netherlands (Table 1). Exclusion criteria were immune disorders, diabetes, usage of immunosuppressive drugs, or fever (>38 °C) within the last month before blood drawing. Inclusion criteria were having received a vaccination with the trivalent influenza vaccine of the 2007–2008 influenza season. Of these individuals a selection was made, based on the expression of HLA-A2 and on the availability of sufficient PBMC (Table 1). Per group, a subset of 5 HLA-A2+ individuals was randomly selected, to determine the effect of adding exogenous recombinant TNF-α to DC cultures of healthy older adults or young adults, respectively.

2.3. Influenza virus

Influenza virus A/Wisconsin/67/2005 (H3N2) grown on MDCK cells (ATCC®, CCL-34™) was generously provided by Dr. Sylvie van der Werf (Institute Pasteur, Paris, France). Medium from uninfected MDCK cells was used as negative control (mock).

2.4. Preparation of monocyte-derived dendritic cells

Human PBMCs were isolated from heparinized whole blood by Lymphoprep (Axis Shield, Oslo, Norway) density centrifugation at $600 \times g$ for 20 min at 20 °C. For the generation of DCs, PBMCs were suspended in culture medium (CM), i.e. IMDM (GIBCO BRL, USA) containing 1% FCS (Hyclone, Utah, USA), penicillin, streptomycin and L-glutamine (Gibco BRL, USA), and were plated at 2.5×10^7 cells/25-mm^2 culture flask (Corning). After 2 h, non-adherent cells were removed by washing with PBS. Adherent cells were incubated for in total 6 days in CM, supplemented with 500 U/ml GM-CSF (Peprotech, London, UK) and 250 U/ml IL-4 (Strathmann GmbH, Hamburg, Germany) to obtain immature dendritic cells.

2.5. Dendritic cell stimulation

Immature DCs were plated at 2×10^5 DCs/well in a 48-wells plate (Greiner Bio-one) in 250 μl CM supplemented with 500 U/ml GM-CSF and 250 U/ml IL-4. DCs were stimulated by incubating with influenza virus A/Wisconsin/67/2005 (H3N2) at a multiplicity of infection (MOI) of 2 for 2 h at 37 °C. As negative control, medium of uninfected MDCK cells was used (mock). After 2 h, an equal amount of medium was added. In addition, 80 ng/ml rTNF-α (InvivoGen, San Diego, USA) was added to the culture where indicated. DCs were incubated for another 4 or 22 h at 37 °C before analysis.

2.6. Detection of dendritic cell maturation and expression of cell surface markers

DC maturation and expression of co-stimulatory molecules were analyzed on a FACS Canto II (BD Biosciences) after staining with anti-HLA class I FITC (clone W6/32, IgG2a), anti-CD86 Pacific Blue (Biolegend, San Diego, USA) and anti-CD80 FITC, anti-CD83 FITC, anti-CD40 PE (BD Biosciences, San Jose, USA).

2.7. Multiplex cytokine analysis

Cytokine production by DCs at 6 and 24 h after stimulation was analyzed by multiplex assay using the Bio-Plex human 27-plex panel containing IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17, Basic FGF, Eotaxin, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF BB, RANTES, TNF-α, VEGF (Biorad, USA) on a luminex 100 (Luminex Corporation, Austin, USA). The multiplex assays were performed according to the manufacturer's protocol.