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Effect of dendritic cell state and antigen-presentation conditions on resulting T-cell phenotypes and Th cytokine profiles

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ABSTRACT

T cells play a pivotal role in controlling the immune response and have been the focus of extensive research. We studied the process of in vitro generation of antigen-specific T effector cells (Teffs) to assess the dynamics of antigen presentation and determine the best conditions for cell therapy. We used a peptidic construct consisting of combined HLA class I and II epitopes of the tumor antigen MAGE-3 as an antigen. Monocytes were isolated from healthy donors and were differentiated to dendritic cells (DCs) in vitro. The peptide was added to the DC culture, the pulsed cells were transferred to a co-culture with lymphocytes from the same donor, either as irradiated feeders or untreated, and were cultured in the presence or absence of IL-2. Several rounds of restimulation followed. The cells were analyzed by Flow Cytometry, and cytokine levels were measured by ELISA and Cytometric Bead Array for Th1/Th2/Th17 profiling. The results showed that the lymphocytes in culture upregulated their activation markers and produced Th1 proinflammatory cytokines in response to the peptide, optimally when it was presented by non-irradiated dendritic cells in the presence of IL-2. In contrast, DC irradiation resulted in low activation potential and a shift toward a suppressive phenotype. After prolonged antigenic stimulation, the culture displayed Th17 polarization. In conclusion, the functional integrity of DCs is necessary for the development of antigen-specific Teffs, and culture conditions can be developed to create Teffs with specific properties for eventual use in cell therapy applications.

1. Introduction

The antigen-presentation process is the first and most critical point during the course of the immune response that defines the type of reaction that will be mounted as well as the response's intensity, extent and duration.

Antigen-presenting cells (APCs) and dendritic cells (DCs) in particular have increasingly been the focus of research during the last decade as orchestrators of the immune response, in contrast to earlier theories that attributed the central role to the specific nature of each antigen (Hart, 1997; Mellman and Steinman, 2001). In view of this concept, the conditions under which the APC encounters an antigen define the signals that will be communicated to the cells of the immune system in its vicinity, mainly naïve T lymphocytes, leading to apoptosis, anergy, tolerance, Th1, Th2 or Th17 responses and finally defining when and to what extent the immune response will be driven to immune regulation or suppression (Matzinger and Kamala, 2011).

During the course of the antigen presentation, the crucial point is the antigen uptake by the professional APCs (mainly DCs), as they solely possess the ability to induce the activation of naïve T lymphocytes (Matzinger, 1994; Mellman and Steinman, 2001). As APCs do not distinguish among the circulating potential antigens in their environment and present them indiscriminately, the conditions prevailing during the antigen uptake and processing will greatly influence the subsequent course of the immune response. Among these conditions are included all the chemical signals present in the cellular microenvironment, indigenous or foreign, protein, lipid or other, as well as any substance produced as a response to the antigenic stimulation. These chemical signals are generally divided in two categories: those that are perceived as "danger signals" and those that belong to the natural repertoire of each tissue (Fuchs and Matzinger, 1996). Among the danger signals are included some components of viruses, bacteria or other microorganisms, such as lipopolysaccharide (LPS) (Abdi et al., 2012), i.e. everything that suggests cellular stress and unprogrammed cellular destruction (Gallucci and Matzinger, 2001), therefore focusing on the damage that the presumed pathogen inflicts on the host, giving the

Abbreviations: APC, antigen presenting cell; CBA, cytometric bead array; CCM, complete culture medium; DC, dendritic cell; MAGE-3, melanoma-associated antigen 3; Teff, T effector cell; Treg, regulatory T-cell.

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danger signal role to proteins normally exclusively located intracellularly, such as proteins bound to DNA fragments and heat shock proteins. Following the recognition of these signals along with the antigens, the APCs initiate a process of expression of co-stimulatory molecules that allows the completion of the antigen presentation to the naïve T lymphocytes by providing both signal 1 (HLA-antigen complex) as well as signal 2 (co-stimulatory molecule) as required (Matzinger and Kamala, 2011). Among the characterized DC-side signal 2 molecules are the surface molecules CD80 and CD86 (or B7.1 and B7.2, respectively) that bind CD28 or CD152 (CTLA-4) on T lymphocytes.

After the initial activation of the naïve T lymphocytes, the cellular and humoral response is polarized according to the signals already received during the above process, and the response subsequently runs its course guided by the local and systemic conditions at the site of the inflammation and in the organism in general. During this stage, the concentration of several key cytokines in the cellular microenvironment, the prolonged antigenic stimulation or even the type of local cellular interactions can shift the immune response to an effector or regulator class or even change the established response to a different one. According to recent findings on T-cell plasticity, several populations, though displaying a terminally differentiated phenotype, retain the ability to switch class in response to exogenous stimuli (O'shea and Paul, 2010; Wan, 2010; Zhu and Paul, 2010).

To summarize, antigen presentation takes place within a set of parameters with little connection to the specific antigen that is central to the process and considerable connection to the type and level of damage inflicted upon the surrounding tissue and according to recent evidence, to the type of the tissue in question. The subsequent course of the inflammation is defined both by this early "programming" and by the conditions that are locally and systemically dominant. These parameters collectively guide the immune system to recruit and differentiate the appropriate class of cells of the innate and the adaptive arm of immunity accordingly resulting in a tailored response to eliminate the pathogen and simultaneously preserve the integrity of the affected tissue.

To investigate the effect of a series of factors on the immune response process, we developed an in vitro system of antigen presentation based on DCs developed from peripheral blood monocytes in co-culture with lymphocytes isolated from healthy individuals. Using this system, we studied the impact of several parameters on the polarization of the T-cell responses in the presence of a non-previously encountered antigen. The detailed evaluation of the effect that exogenous factors exert on the antigen-presentation system will allow us to eventually manipulate the process to shift it toward the generation of the desired T-cell polarization according to the application in question.

2. Materials and methods

2.1. Antigenic peptide

As a model antigen, we selected a construct combining a CD4-HLA II and a CD8-HLA I epitope mapping to the cancer antigen MAGE-3, mounted on a synthetic helicoid-type sequential oligopeptide carrier (SOC_4) as previously described (Celis et al., 1994a,b; Consogno, 2003; Dimtsoudi et al., 2006; Kargakis et al., 2007; Krikorian et al., 2005; Manici et al., 1999; Sakarellos-Daitsiotis et al., 2006).

2.2. Development of DCs from peripheral blood mononuclear cells

Samples of heparinised blood (10–15 ml) were drawn from 5 healthy individuals (3F/2 M, age range 22–30 years). Informed consent was obtained from each person, in accordance to the Helsinki declaration on ethical principles for medical research involving human subjects.

DCs were developed in vitro as previously described (Sallusto and Lanzavecchia, 1994). Briefly, peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation over a Ficoll-Paque gradient (Pharmacia, Sweden). The cells were resuspended in RPMI-1640 culture medium (Gibco-BRL, Gaithersburg, MD, USA) containing 10% fetal bovine serum (Gibco-BRL), 50 IU/mL penicillin, 100 μg/ml streptomycin, 5×10^{-5} mol/L mercaptoethanol (Sigma, St. Louis, Mo, USA) (complete culture medium, CCM) at a concentration of 5×10^6 cells/mL and were incubated for 2 h in plastic-bottom culture plates. Subsequently, the non-adherent cells were removed by gentle agitation and aspiration and were centrifuged and resuspended in CCM and stored at 4 °C for 8 days until their re-addition to the culture. Fresh CCM enriched with IL-4 (1000 IU/ml, R&D Systems, Minneapolis, MN, USA) and GM-CSF (1000 IU/ml, R&D Systems) (enriched CCM) was added to the adherent cells. The cells were resupplied with enriched CCM every 48 h for 6 days. On day 6, the cultured DCs were incubated for 2 h in the presence of the antigenic peptide MAGE-3 at a concentration of 10 μg/ml, and afterward 0.25 μg/ml lipopolysaccharide (LPS, Sigma) was added in the culture. The cells were resupplied with enriched CCM and were cultured for an additional 48 h. When irradiation was required, the cells received a total amount of 25 Gy on the 8th day of culture and were subsequently resupplied with fresh CCM (Fig. 1).

2.3. Antigen-presentation system

For each experiment, the non-adherent PBMCs that had been stored at 4 °C were tested for viability by trypan blue staining and in each case the viability was confirmed to be >90%. The percentage of CD3+ T lymphocytes in the stored cells was >50% in all samples. The cells were subsequently placed in co-culture with the antigen-pulsed DCs in the presence of IL-12 (2 IU/ml, R&D Systems). For each round of stimulation, the antigen was added to the culture at 11-day intervals at a concentration of 1 μg/ml, together with IL-12 (2 IU/ml). On the 4th and 7th day after each activation, IL-2 (R&D Systems) was added according to the experimental conditions at a concentration of 25IU/ml (Fig. 1). All experimental points were repeated in triplicate.

2.4. Immunophenotyping of cultured cells

At specific time points (as indicated in Section 3), cells were removed from the culture and were incubated with the mAbs CD4-FITC (Beckman Coulter (BC), France), CD8-FITC (BC), CD20-FITC (BC), CD45RO-PE (Becton Dickinson Biosciences/Pharmingen (BD), San Diego, CA, USA), HLA-DR-PE (BD), CD45RA PE (BD), CD127-PE (BC), CD56-PE (BC), CD25-PC5 (BC), CD25-PE (BD), CD3-PC5 (BC), FoxP3-PE-Cy5 (eBioscience, San Diego, CA, USA), CD69-PE (BD), CD86-PE (BD), CD83-PE (BD), CD38-PE (BD) and CD14-FITC (BD). All experimental procedures were conducted following the manufacturers' instructions for cultured PBMCs. Negative controls consisted of isotype-matched irrelevant antibodies that substituted specific antibodies at equivalent concentrations. Flow cytometric acquisition and analysis were performed on at least 10,000 acquired events (gated on lymphocytes) per sample, using a Coulter EPICS-XL-MCL cytometer (BC). The data were analyzed using the FlowJo V7.5 software (Tree Star Inc., Ashland, OR, USA).

2.5. Determination of culture supernatant cytokine levels

Measurement of the concentration of the cytokines IL-2, IL-4, IL-5, IL-6, IL-17, IL-10, TNF-α and IFN-γ in culture supernatants was performed with a BD FACSArrayBioanalyzer, using a cytometric