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Immunisation with an allogeneic peptide promotes the induction of antigen-specific MHC II^{pos} CD4^{+} rat T cells demonstrating immunostimulatory properties

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ABSTRACT

Background: The phenomenon of T cell stimulation by MHC class II expressing (MHC II^{pos}) CD4^{+} T cells has been intensively investigated for T cell clones but, so far, not for native T cells. The extensive use of T cell clones may explain the inconsistent outcomes of T cell-mediated antigen-presentation. Therefore, we used freshly isolated primed rat CD4^{+} T cells induced by immunisation with an allogeneic peptide P1, which is involved in allograft rejection.
Methods: MHC II^{pos} and MHC II^{neg} CD4^{+} T cells were isolated from popliteal lymph nodes of P1-immunised Lewis rats and were purified by combining depletion and positive selection steps. Purified MHC II^{pos} CD4^{+} T cells and MHC II^{neg} CD4^{+} T cells (10^5 cells per well each) were autostimulated or restimulated with P1-loaded (33 μg/ml peptide P1) and subsequently irradiated (with 20 Gy) autologous DC.
Results: Seven days after immunisation, a small population of MHC II^{pos} CD4^{+} T cells was detectable (approximately 8.0% of total lymph node cells), as well as a large population of MHC II^{neg} CD4^{+} T cells (up to 45%). Antigen-specific proliferation was observed for both T cell populations but only P1-loaded MHC II^{pos} CD4^{+} T cells presented antigen presenting cell (APC) function for P1-primed T cells. Their inability to activate unprimed T cells may be due to impaired surface expression of costimulatory molecules (CD80 and CD86).
Conclusion: Immunisation with the allogeneic peptide antigen P1 induced antigen-specific MHC II^{pos} CD4^{+} rat T cells demonstrating perfect APC function for primed T cells *in vitro*.

1. Introduction

The MHC class II-mediated antigen presentation by professional antigen presenting cells such as dendritic cells (DC), macrophages and B cells, is an important prerequisite to triggering T cell-mediated immune responses. In contrast, MHC class II expression by nonprofessional APC, e.g. fibroblasts, epithelial cells, that is induced by proinflammatory cytokines (IFN-γ, TNF-α), mediates T cell-apoptosis which is thought to prevent accumulation of antigen-specific activated T cells after removal of the initiating antigen [1]. Proper T cell activation requires not only the interaction between T cell receptor and MHC class II/peptide complex on APC, but according to the two signal concept of T cell activation, costimulatory signals are also required. Delivery of antigenic signals through the T cell receptor in the absence of proper antigen-independent costimulatory signals can induce a state of unresponsiveness in these T cells upon antigenic restimulation [1]. Therefore, it is of particular interest that activated T cells express costimulatory molecules at lower levels than professional APC [2].

MHC class II surface expression on T cells was shown for different species, e.g. rat [3], human [4,5], cow [6], horse [7], and rabbit [8]. The master regulator of MHC class II gene expression is the class II transactivator (CIITA) and its expression was shown for human [9] and rat [10–13] activated T cells. The CIITA gene expression is controlled by at least three independent promoter units: CIITA-P1, CIITA-PIII and CITTA PIV [14]. The expression of MHC class II molecules on murine T cells has been discussed controversially but presently it is generally accepted that murine T cells do not express MHC class II molecules. Antigen presentation by mouse T cells is mediated by the acquisition of peptide-loaded MHC class II molecules [15–17].

In the late 1970s, different laboratories demonstrated that circulating human peripheral blood T cells can express MHC class II determinants and these MHC II^{pos} CD4^{+} T cells demonstrated APC function

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by presenting antigens to autologous primed T cells [4,5]. However, even after more than 30 years of investigation, the precise role of T cell-mediated antigen-presentation and T cell-activation in immune responses remains a matter of debate [18]. Most of the studies published describe the induction of nonresponsiveness (anergy or apoptosis) in responder T cells [2,19], whereas some authors see the role of antigen presentation by activated T cells in augmenting the immune response [20].

Most of the information on the outcome of T cell-mediated antigen-presentation is available for T cell lines [21–23]. To determine the impact of T cell-mediated antigen presentation and T cell-mediated immune response on allograft rejection, T cells from popliteal lymph nodes of P1-immunised Lewis rats were purified by combined depletion and positive selection steps. We reported in previous studies that the immunisation with the allogeneic peptide P1 [24,25] induced antigen-specific $CD4^+$ T cells that are involved in allograft rejection in the allogeneic donor–recipient rat strain combination Wistar Furth-to-Lewis [26].

2. Objectives

In the present study the effect of antigen presentation by MHC II^{pos} $CD4^+$ T cells on T cell proliferation, cytokine production and surface molecule expression was analysed in comparison to the DC-mediated effects.

3. Materials and methods

3.1. Animals

Male rats of the inbred Lewis strain ($RT1^l$ haplotype) and Wistar Furt strain ($RT1^u$ haplotype) weighing 150–250 g were used (Harlan Europe, The Netherlands). The study was reviewed and approved by the Animal Care Committee of the local government in accordance with the European and national guidelines for animal care (German Law for the Protection of Animals).

3.2. Peptides and immunisation

The MHC class II ($RT1.B^l$)-binding peptide P1 was based on a published sequence [27] and synthesised with a purity of about 95% at Jerini AG (Berlin, Germany). The immunodominant peptide P1 is a strong activator of an alloreactive T cell response and P1-immunised Lewis rats reject accelerated Wistar Furth cardiac allografts [24,26]. The MHC class II ($RT1.B^l$)-binding peptide CP was used as a control peptide [24]. Antigen-specific T cells were induced by immunising Lewis rats subcutaneously in each hind footpad with 50 μg of the allogeneic peptide P1 (1 mg/ml stock solution) emulsified with the same volume of adjuvant TiterMax (Enzo Life Sciences GmbH, Germany) as described previously [25]. Lymph node cells were isolated seven days after immunisation.

3.3. Cell isolations

Splenic DC were isolated from nonimmunised Lewis rats and lymph node T cells from nonimmunised Wistar Furth rats as described previously [26]. MHC II^{pos} and MHC II^{neg} $CD4^+$ T cells were isolated from popliteal lymph nodes of P1-immunised Lewis rats and were purified by combining depletion and positive selection steps. First, adherent cells were depleted by culturing the popliteal lymph node cells in plastic dishes for 2 h. Then, $CD8^+$ T cells and B cells were depleted from suspension cells by incubating them in saturating concentrations (10 μg/ml) of the anti-CD8 antibody Ox8 and anti-B cell antibody Ox33 for 30 min at 4 °C. Subsequently, $CD4^+$ T cells (2×10^7 cells/ml RPMI 1640) were positively selected with the anti-TCR α/β antibody R73 (0.5 μg antibody per 1×10^6 target cells). After 20 min of incubation at 4 °C, the cells were washed two times with medium, 5 magnetic beads (CellLection pan mouse IgG kit, Invitrogen, DYNAL) per target cell were added, and cells and magnetic beads were incubated for 20 min. The tubes were placed in the magnetic particle concentrator (Invitrogen, DYNAL) for 1 min, unbound cells were discarded with the supernatant and the bead-bound cells were washed 3 times with buffer. The addition of DNase I (50 U/μl) released the R73-positive T cells from the beads by cleavage of the DNA linker between bead and antibody. On the next day MHC II^{pos} $CD4^+$ T cells were enriched with the anti-RT1.B antibody Ox6 [28] in a further positive isolation step. The purity of MHC II^{pos} and MHC II^{neg} $CD4^+$ T cells (>95%) was confirmed by flow cytometry. All antibodies were purchased from Serotec, Ltd, Oxford, United Kingdom.

3.4. T cell proliferation and suppression assay

Popliteale lymph node cells, purified MHC II^{pos} $CD4^+$ T cells and MHC II^{neg} $CD4^+$ T cells (10^5 cells per well each) were used as responder cells. They were stimulated with with two types of P1-loaded (33 μg/ml peptide P1) stimulator cells: autologous DC and MHC II^{pos} $CD4^+$ T cells (10^4 cells per well). The stimulator cells were subsequently irradiated with 20 Gy and cultured in a final volume of 150 μl RPMI 1640 medium supplemented with 10% FCS, 25 mM HEPES, 2 mM glutamine, 50 μM 2-mercaptoethanol, 1 mM sodium pyruvate, 100 IU/ml penicillin, and 0.1 mg/ml streptomycin. The 96-well, round-bottom plates were incubated for 3 days at 37 °C in a 5% humidified CO_2 atmosphere. T cell proliferation was measured after [^{3}H]-thymidine (0.5 μCi/well) incubation for the last 6 h before harvesting. Radioactivity was determined as previously described [24]. Results (mean ± standard deviation) were expressed in counts per minutes (cpm).

3.5. Flow cytometric analysis

For flow cytometric analysis, 5×10^5 cells each were incubated in 50 μl PBS for 20 min at 4 °C with the following antibodies: Ox6 (anti-MHC class II); 3H5 (anti-CD80), and 24F (anti-CD86) [29]. The antibodies 3H5 and 24F were purchased from BD Biosciences, Heidelberg, Germany. Nonviable cells were identified with 7-amino actinomycin D (BD Biosciences). The samples were analysed with the flow cytometer FACSscan (BD Biosciences). Data were acquired with CellQuest Pro software and the analyses performed with WinMDI 2.9 software (http//facs.scripps.edu/software.html).

3.6. Reverse transcriptase polymerase chain-reaction (RT-PCR)

One million cells were lysed in Trizol (Invitrogen Life Technologies GmbH, Karlsruhe, Germany) and the RNA was extracted according to the manufacturer's recommendations, as described previously [26]. Reverse transcription of 1 μg total RNA was performed with reverse-transcriptase (2.5 U/μl) and Oligo $d(T)_{16}$ primer (2.5 μmol/l), using the GeneAmp RNA-PCR-Kit (Applied Biosystems GmbH, Germany). One μl cDNA was amplified using Gold AmpliTaq DNA-Polymerase (0.05 U/μl) and 5 μmol/l each specific primers (Table 1) mixed in nuclease free water (Promega GmbH, Germany). The housekeeping gene Glycerinaldehyd-3-Phosphat-Dehydrogenase (GAPDH) was used as positive control, PCR products were analysed by electrophoresis on 2% agarose gels. In addition, purified PCR products were sequenced to confirm their accuracy (SeqLab GmbH, Göttingen, Germany).

4. Results

4.1. Immunisation with the allogeneic peptide P1 induces antigen-specific lymph node T cells

Seven days after subcutaneous immunisation with the allogeneic peptide P1 [24], the drained popliteal lymph nodes were enlarged and the number of popliteal lymph node cells had significantly increased from