Ph.D. THESIS

ROLE OF NITRIC-OXIDE ON T CELL ACTIVATION

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Inroduction

The signaling networks that mediate activation, proliferation, or programmed cell death of T lymphocytes are dependent on complex redox and metabolic pathways.

TCR signaling is initiated when antigen presented by major histocompatibility complex (MHC) molecules binds to the TCR complex. Signaling via the TCR has been extensively studied using anti-TCR or anti-CD3 monoclonal antibodies, which bind to and activate the receptor similar to MHC-presented antigen. Engagement of the TCR leads to activation of Src family kinases Lck and Fyn. Lck then phosphorylates the immunoreceptor tyrosine-based activation motifs (ITAM) contained within the TCR chains. Subsequent recruitment of ZAP-70 to the phosphorylated ITAM initiates a cascade of tyrosine phosphorylation events, that promotes the activation of ZAP-70-induced tyrosine phosphorylation, include LAT protein. Phosphorylated LAT binds directly to phospholipase C-γ-1. Further downstream, phospholipase C-γ-1 controls hydrolysis of phosphatydilinosito4,5-biphosphate to inositol-1,4,5-triphosphate (IP3) and diacylglycerol. IP3 binds to its receptors in the endoplasmic reticulum, opening Ca^{2+} channels that release Ca^{2+} to the cytosol. Sustained increase of intracellular Ca^{2+} levels mediates coupling of ATP production to metabolic need during T cell activation.

The mitochondrion, the site of oxidative phosphorylation, has long been identified as a source of energy and cell survival. The synthesis of ATP is driven by an electrochemical gradient across the inner

mitochondrial membrane maintained by an electron transport chain and the membrane potential (negative inside and positive outside). A small fraction of electrons react directly with oxygen and form reactive oxygen intermediates (ROI). Although ROI have long been considered as toxic by-products of aerobic existence, evidence is now accumulating that controlled levels of ROI modulate various aspects of cellular function and are necessary for signal transduction pathways, including those mediating apoptosis. Mitochondrial ROI production modulates T cell activation, cytokine production, and proliferation at multiple levels. Elevation of the mitochondrial transmembrane potential or mitochondrial hyperpolarization has been recently identified as an early event associated with apoptosis and T cell activation.

Following exposure to NO, persistent mitochondrial hyperpolarization was recently observed in astrocytes. Elevation of mitochondral membrane potential is also triggered by activation of the CD3/CD28 complex or stimulation by ConA. Therefore, mitochondrial hyperpolarization represent an early but reversible switch not exclusively associated with apoptosis. Thus, mitochondrial hyperpolarization is a likely cause of ROI production at early stages of T cell activation and apoptosis.

Once activated, CD4 T cells proliferate and differentiate into two main subsets of primary effector cells, Th1 or Th2 cells, characterized by their specific cytokine expression pattern. Th1 cells promote cellular immunity and macrophage activation largely through the production of interleukin-2 (IL-2) and interferon-gamma

(IFN-γ). Th2 cells, through the expression of IL-4, IL-5, and IL-13, induce IgE production by B cells and eosinophil-mediated and mast-cell-mediated immune responses, and orchestrate the defense against extracellular parasites. Th2 cells have a central role in driving the immune response in asthma and atopic diseases. The Th1/Th2 balance is therefore considered to be pivotal in chronic inflammatory diseases.

Histamine selectively enhances the secretion of Th2 cytokines such as IL-4, IL-5, IL-10 and IL-13 and inhibits the production of Th1 cytokines IL-2 and IFN-γ and monokine IL-12. The crucial role of histamine in the early events of the pathogenesis of atopic asthma is associated with the increased production of Th2 and decreased production of Th1 cytokines.

In contrast to histamine, NO selectively enhances Th1 cell proliferation and represents an additional signal for the induction of T cell subset response. Although there were several pharmacological approaches to study the biological role of histamine in allergic and autoimmune diseases, its exact role in immune regulation is far from being uncovered. It has been shown that both histamine and NO can modulate the cytokine network in multiple ways, however, the possible role of NO in the immunoregulatory functions of histamine is not known. We also investigated the effect of genetically induced histamine deficiency on T lymphocyte cytokine expression and T cell activation, using HDC gene knockout (HDC-KO) and congenic wild-type mouse. Our results indicate that histamine deficiency profoundly alters T lymphocyte cytokine expression and T cell

activation. Furthermore, our data show that in addition to its direct effects on T cells, histamine modulates immune responses through regulating NO production.

Specific aims

To investigate the molecular ordering of T cell activation-induced cytoplasmic and mitochonadrial signals that lead to mitochondrial hyperpolarization.

To study the effect of IP3 antagonist 2-APB, NO chelator C-PTIO and superoxide dismutase mimetic MnTBAP on T cell activation.

To investigate the effect of NO donor on the intracellular Ca^{2+} level, ROI production and mitochondrial hyperpolarization.

To study the expression of NOS isoforms in resting and CD3/CD28 stimulated lymphocytes.

Histamine deficient T cells were used to study the possible interaction of NO and histamine in T cell activation and cytokine production.

Methods

Cell culture

PBMC were isolated from heparinized venous blood on Ficoll-Hypaque gradient. PBL were resuspended at 10^6 cells/ml in RPMI 1640 medium, supplemented with 10% FCS, 2 mM Lglutamine, 100 IU/ml penicillin, and 100 μg/ml gentamicin in 12-well plates at 37°C in a humidified atmosphere with 5% CO2.

Animals

The strategy to generate HDC-KO mice has been described previously. HDC-KO CD1 mice were backcrossed onto the BALB/c background over nine generations. Male mice were 6–8 weeks of age at the beginning of the sensitization. All mice were maintained on histamine-free diet. In some experiments wild type and HDC-KO mice were injected with complete Freund's adjuvant (CFA) and splenocytes were isolated 9 days later. Splenocytes were resuspended at 10^6 cells/ml in complet RPMI 1640 medium.

Measurement of intracellular NO levels, and serum nitrite-nitrate level

Production of NO was assessed by using DAF-FM or a NO sensor kit. Excitation and emission maxima of DAF-FM are 495 and 515 nm, respectively. C-PTIO (500 μM), specific NO chelator, was used to reduce NO levels and inhibit NO signaling. Serum nitrate/nitrite levels were meausured by using High-Sensitivity Nitrite Assay Kit.

Flow cytometric analysis of mitochondrial mass and ROI production.

Mitochondrial mass was monitored by staining with 50 nM nonyl acridine orange (NAO, excitation, 490 nm; emission, 540 nm recorded in FL-1). Fluorescent probe was obtained from Molecular Probes. ROI was monitored by 1 μM hydroethidine (HE). Fluorescence emission from oxidized HE, was detected at a wavelength of 560 nm.

Intracellular flow cytometry

Splenocytes form HDC-KO and wild type mice were stimulated with 2 μg/ml ConA for 48h, treated with GolgiPlug™ Protein Transport Inhibitor (contains Brefeldin A). Than 0.5 μg/ml fluorochrome-conjugated monoclonal antibody specific for cell surface antigens (CD3-PE, CD4-PerCP, CD8-PerCP, CD45-PerCP or CD25-PerCP-CY5) were added. Cells were washed in PBS then fixed and permeabilized. Cells were washed, then incubated 0,5 μg/ml FITC-conjugated anti-INF-γ. Samples were analyzed by three or four colour analysis on a FACSCalibur flow cytometer using CELLQuest software version 3.1.

Western blot analysis of NO synthase (NOS) expression

PBL were washed in PBS and resuspended in NOS solubilization buffer. After freezing and thawing thrice, the sample was pelleted by centrifugation, and the supernatant was used in subsequent experiments. Twenty micrograms of protein lysates were separated on a 7.5% SDS-polyacrylamide gel and electroblotted to nitrocellulose. Nitrocellulose strips were probed with NOS isoform-specific rabbit Abs. Expression of NOS isoforms was visualized by ECL followed by detection of β-actin using 4-chloronaphthol. Automated densitometry was used to quantify the relative levels of protein expression using a Kodak Image Station 440CF with Kodak 1D Image Analysis software.

ELISPOT

Splenocytes were plated in duplicates in 96-well plates (10^5 cells/plate) precoated with an anti-IFN-γ, anti-IL-10 or anti-IL-4 capture monoclonal antibody. Cells were stimulated with 2 μg/ml

Con-A for 24 hours. After washing with PBS-Tween, the cells were incubated with biotinylated anti-IFN-γ, anti IL-10 or anti IL-4 detection mAb. Than cells were incubated with streptavidin-HRP conjugate, and then, after further washes incubated by the chromogenic substrate (aminoethyl carbazole). The plates were washed with destilleted water. The number of IFN-γ, IL-10 or IL-4 producing spot forming cells was counted by ELISPOT reader.

Quantitative RT-PCR

T cells were isolated by using magnetic beads (negative selection), total RNA was extracted from T cells using RNeasy® Mini Kit. First strand cDNA was produced using random hexamers. Relative quantification of nNOS, iNOS, and eNOS mRNAs was performed with a TaqMan real-time RT-PCR assay on an ABI PRISM 7000 Sequence Detector using standard protocols. All reactions were run in duplicate and included no template and no reverse transcription controls for each gene. Analyses of real-time quantitative PCR data were performed using the comparative threshold cycle (CT) method. The relative amount of mRNA was referred to the one of hypoxanthine phosphoribosyl transferase (HGPRT).

Statistics

Results were analyzed by Student's t test or Mann-Whitney U rank sum test for nonparametric data. Changes were considered significant at $p < 0.05$.

Results

CD3/CD28 costimulation elicits elevation of intracellular and mitochondrial Ca^{2+} levels, ROI and NO production, and mitochondrial hyperpolarization

Mitochondrial hyperpolarization represents an early and reversible checkpoint associated with both T cell activation and apoptosis signals. In thepresent study, we investigated the contribution of these T cell activation signals to mitochondrial hyperpolarization. CD3/CD28 costimulation of human T lymphocytes increased cytoplasmic Ca^{2+} levels, by 25% after 20 min ($p < 0.03$), followed by further rises, 3.9 ± 0.75-fold after 4 h ($p = 0.001$) and 2.77 ± 0.75-fold after 24 h ($p = 0.0043$). In parallel, mitochondrial Ca^{2+} levels increased by 22% after 20 min ($p = 0.009$), 2.15 ± 0.5-fold after 4 h ($p = 0.001$), and 2.78 ± 0.8-fold after 24 h ($p = 0.001$), respectively. In comparison to baseline, cytoplasmic ROI levels increased to 1.32 ± 0.36-fold 20 min ($p = 0.026$), 1.53 ± 0.36-fold ($p = 0.002$) 4 h, and 4.57 ± 1.75-fold ($p = 0.001$) 24 h after T cell stimulation. NO levels increased 6.09 ± 2.98-fold 4 h ($p = 0.001$) and 4.9 ± 1.8-fold 24 h after T cell stimulation ($p = 0.001$). Mitochondrial membrane potential was elevated 1.73 ± 0.44-fold ($p = 0.001$) 4 h and 1.53 ± 0.17-fold ($p = 0.001$) 24 h after CD3/CD28 stimulation. Although monocyte-depleted PBL were used for stimulation of CD3/CD28, we investigated whether NO was produced by T cells.

IP3R antagonist 2-APB and NO chelator C-PTIO inhibit CD3/CD28 costimulation-induced mitochondrial hyperpolarization

The membranepermeant IP3R inhibitor 2-APB (100 μM) reduced elevation of cytoplasmic Ca^{2+}, NO production, and mitochondrial hyperpolarization 4 h after CD3/CD28 costimulation. 2-APB also diminished late ROI production. C-PTIO (500 μM), specific NO chelator, reduced NO levels (-80 ± 7.5%; p = 0.004) and profoundly inhibited mitochondrial hyperpolarization (-85.0 ± 10.0%; p = 0.008), ROI production (-88 ± 19%; p = 0.01), and cytoplasmic (-75 ± 5.6%; p = 0.001) and mitochondrial Ca^{2+} elevation (-62 ± 10.0%; p = 0.005).

NO induces coordinate elevation of cytosolic and mitochondrial Ca^{2+} levels, ROI production, and mitochondrial hyperpolarization

Both calcium ionophore ionomycin and calcium ATPase inhibitor thapsigargin markedly increased cytoplasmic and mitochondrial Ca^{2+} levels. Neither ionomycin and nor thapsigargin affected mitochondrial membrane potential or NO production. ROI production was increased 1.32 ± 0.16-fold (p = 0.013) by thapsigargin. Effect of NO on mitochondrial membrane potential was evaluated by exposing PBL to NO donors NOC-18 Treatment of human PBL with 600 □M NOC-18, capable of slowly releasing NO, increased DAF-FM fluorescence by 3.13 ± 0.8-fold after 4 h (p = 0.04) and 3.7 ± 0.6-fold after 24 h (p = 0.03).NOC-18 also enhanced cytoplasmic and mitochondrial Ca^{2+} as well as ROI levels.

Raising Ca^{2+} levels by ionomycin and thapsigargin failed to elicit sustained mitochondrial hyperpolarization. Interruption of T cell activation-induced Ca^{2+} signaling with the membrane-permeant IP3R inhibitor 2-APB reduced elevation of cytoplasmic Ca^{2+}, and NO and

ROI production. C-PTIO, specific NO chelator exhibited the inhibitory effect on T cell activation-induced NO productions (-80 ± 7.5%; p = 0.004), mitochondrial hyperpolarization (-85.0 ± 10.0%; p = 0.008), ROI production (-88 ± 19%; p = 0.01), and cytoplasmic (-75 ± 5.6%; p = 0.001) and mitochondrial Ca^{2+} elevation (-62 ± 10.0%; p = 0.005). Furthermore, inhibition of T cell activation-induced Ca^{2+} signaling by 2-APB was less effective than NO chelator C-PTIO in blocking mitochondrial membrane potentail elevation. Although ROI quenching by MnTBAP and DIPPMPO reduced ROI production and, to a lesser extent, NO production, they failed to influence mitochondrial membrane potential elevation. These results suggested that T cell activation-induced ROI and Ca^{2+} signals contribute to NO production, with the latter representing a final and dominant step in mitochondrial hyperpolarization.

Expression of eNOS and nNOS is enhanced by CD3/CD28

Western blot analysis of protein lysates from human PBL revealed expression of eNOS and nNOS and absence of iNOS. With respect to β-actin, eNOS and nNOS protein levels were stimulated up to 15-fold by CD3/CD28 costimulation. Expression of eNOS and nNOS was also enhanced by treatment with 100 μM H_2O_2. Ionomycin or thapsigargin did not affect expression of eNOS and nNOS, suggesting that T cell activation-induced NOS expression was delayed and depended on production of H_2O_2 and ROI. Indeed, CD3/CD28 costimulation-induced NO production was inhibited by superoxide dismutase mimic MnTBAP and ROI spin trap DIPPMPO.

HDC-KO splenocytes produce increased IFN-γ at both mRNA and protein levels

Previous data from our laboratory reveal that HDC-KO mice are characterized by a strong Th1-biased cytokine pattern. Since IFN-γ is a prototype of Th1 cytokines, we first studied IFN-γ production of HDC-KO and wild type splenocytes. Splenocyte IFN-γ mRNA levels were measured by RT-PCR. In comparison with wild type splenocytes, HDC-KO splenocytes displayed significantly higher levels of IFN-γ mRNA ($p<0.001$). To further study cytokine production of HDC-KO and wild type splenocytes, IFN-γ, IL-4 and IL-10 protein levels were measured by EISPOT method, following in vitro stimulation with 2 μg/ml Con-A. In accordance with the PCR data, the IFN-γ production of splenocytes from HDC-KO mice was higher than that of the wild type animals ($p<0.001$). IL-4 and IL-10 protein levels were similar in both HDC-KO and wild type splenocytes ($p=0.23$ and $p= 0,4$ respectively). The IFN-γ production of the CD4, CD4/CD25, CD8, CD45 positive T cell subsets were measured by intracellular flow cytometry. IFN-γ production was similar in all these subsets of both the HDC-KO and wild type T cells. The increased IFN-γ gamma production seems to represent a shift in the entire T cell population.

Histamine regulates NO production in T cells

NO is an important physiological regulator of T cell function, thus, we next studied NO synthase (NOS) expression and NO production of T lymphocytes derived from HDC-KO and wild type mice. Following isolation of T cells from splenocytes, NOS expression of

the HDC-KO and wild type T lymphocytes was measured by quantitative real time RT-PCR. Our data indicate the highest expression of the neuronal (nNOS) isoform, while lower level of inducible (iNOS) and endothelial (eNOS) isoforms were detected. Although all three isoforms were expressed predominantly in the HDC-KO T cells, there was no significant difference in the expression of NOS isoforms between the wild type and HDC-KO T cells. The NO production of T lymphocytes was measured by flow cytometry. T cells from HDC-KO mice produced higher amounts of NO than those from the wild type ($p=0.0009$). T cell activation is associated with ROI and NO production. In addition to higher baseline NO production of the T lymphocytes of HDC-KO animals, T cell activation elicited accelerated NO signal ($p=0.00024$). To further investigate the role of histamine in the regulation of NO production, HDC-KO and wild type splenocytes were costimulated with 2 μg/ml ConA and 10-6 M histamine for 24 hours, and the NO production of T cells was measured by flow cytometry. According to our data, histamine downregulates NO production of both HDC-KO and wild type T lymphocytes ($p=0.0004$ and $p<0.001$, respectively). Nitrite and nitrate are stable end products of NO production. Our data indicate that T cells from HDC-KO mice produce higher amounts of NO than control T cells, but a substantial amount of NO can be generated by many other cell types as well. To estimate total NO production, the serum nitrite and nitrate concentrations of HDC-KO and wild type mice were measured. Nitrite and nitrate levels were measured both following in vivo CFA treatment and without in

vivo stimulation. There was no significant difference in the nitrite and nitrate productions between the HDC-KO and wild type animals in either conditions.

T cell activation-induced rapid Ca^{2+} signal is accelerated in HDC-KO T cells

Our previous data show that NO regulates the activation and signal transduction of T cells, therefore we next investigated the T cell activation-induced Ca^{2+}-signal in HDC-KO and wild type T lymphocytes. Activation of T cells through the TCR initiates a biphasic elevation in the cytosolic free Ca^{2+} concentration, a rapid initial peak observed within 5–10 min, and a plateau phase lasting 4h to 48 h. Cytoplasmic Ca^{2+} concentration of unstimulated T cells and T cell activation-induced rapid Ca^{2+}-signal are both markedly increased in HDC-KO T cells ($p=0.02$; $p=0.04$ respectively). Indeed, although the basal Ca^{2+} level is higher in the HDC-KO T cells, there is no difference in the stimulation induced delta Ca^{2+} levels. T cell activation-induced sustained Ca^{2+}-signal was similar both in HDC-KO and wild type T lymphocytes. To investigate the role intracellular Ca^{2+} on IFN-γ production, the effect of cell permeable intracellular Ca^{2+} chelator BAPTA-AM was studied. Con-A treatment induced IFN-γ production was inhibited by 10 μM BAPTA-AM cotreatment ($p=0.001$).

NO regulates IFN-γ production

Our present data indicate that the increased NO production of HDC-KO T cells is associated with altered cytokine production and T cell signal transduction. Since NO may modulate gene transcription and

cytokine production, next we investigated if NO directly regulates IFN-γ . production. NO precursor (Z)-1-[2-(2-aminoethyl)-N-(2-ammonioethyl)amino]diazen-1-ium-1,2-diolate diethylenetriamine (NOC-18) releases NO in a dose dependent manner. Treatment of splenocytes with NO precursor NOC-18 (60 μM for 24h) increased 2 μg/ml ConA- induced IFN-γ production (1.38±0.13 fold; p=0.0002). 600 μM nitronidazole and 100 μM NG-Monomethyl-L-arginine (LNMMA) both inhibited IFN-γ production of HDC-KO splenocytes (p=0.01; p=0.002, respectively). In parallel, NO production, monitored by DAF-FM fluorescence, was inhibited by more than 60 percent, following both LNMMA and nitronidazole treatment. Moreover, pharmocological inhibition of NO production in HDC-KO splenocytes decreased IFN-γ synthesis, further supporting the role of NO in regulating IFN-γ production. By contrast, treatment of splenocytes with 10-6M histamine did not significantly alter 2 μg/ml ConA-induced IFN-γ production. There was no significant difference in the L-histidine serum levels of the HDC-KO and wild type mice (73±18μM; 64±13μM respectively, p=0.39). Furthermore, L-histidine treatment did not alter Con-A induced IFN-γ production (p=0.3), measured by ELISPOT assay.

Mitochondrial biogenesis in HDC-KO and wild type T cells

NO has recently been recognized as a key signal of mitochondrial biogenesis. Mitochondria can take up, store, and release Ca^{2+} thus altered mitochondrial mass have a role in shaping Ca^{2+} signal in many cell types, including T lymphocytes. Since HDC-KO T cells produce higher amounts of NO, mitochondrial mass was measured in

both HDC-KO and wild type T cells. Our data indicate that there is no significant difference between the mitochondrial mass of HDC-KO and wild type T cells (p=0.1).

ROI production and CD3 internalization in HDC -KO and wild type T cells

Since ROI production is associated with T cell activation, and both Ca^{2+} flux and NO production are different in HDC-KO T cells, next we investigated constitutive and activation- induced ROI production. Our data indicate that basal ROI production and 4h Con-A stimulation-induced ROI production are similar both in the HDC-KO and the wild type T cells. However, the 24h Con-A treatment induced ROI signal was smaller in the HDC-KO T cells. This is in accordance with our previous data, indicating, that NO regulates T cell activation-induced ROI signal. The T cell activation-induced CD3 internalization was similar in both HDC-KO and wild type T lymphocytes.

Conclusions

NO is recognized as an important intercellular and intracellular messenger; however, its role in T cell activation has not been established. There are three known isoforms of NOS: nNOS, eNOS, and iNOS. Expression of eNOS has been previously demonstrated in human peripheral blood B and T lymphocytes, whereas TCR activation was found to induce expression of nNOS by ZB4 murine T cell hybridoma cells. Western blot analysis revealed expression of eNOS and nNOS and absence of detectable iNOS in control and CD3/CD28-costimulated PBL. Unlike iNOS, eNOS and nNOS are

inactive at baseline Ca^{2+} levels. Inhibition ofT cell activation-induced NO production via interference of Ca^{2+} signaling by 2-APB is also consistent with involvement of eNOS or nNOS. Treatment with NO donor NOC-18 or CD3/CD28 costimulation resulted in similar patterns of transient ATP depletion, resulting in a transiently increased susceptibility to cell death via necrosis. NO-induced ROI production may also facilitate necrosis via oxidation of cysteine residues in the active sites of caspases. ROI mediate signaling through the CD3/CD28 receptors. Endogenous H_2O_2 is generated by superoxide dismutase from ROIs in mitochondria. In turn, H_2O_2 is scavenged by catalase and glutathione peroxidase. Although H2O2 is freely diffusible, it has no unpaired electrons and, by itself, is not a ROI. Induction of apoptosis by H_2O_2 requires mitochondrial transformation into an ROI, through the Fenton reaction. In accordance with previous studies. H_2O_2 elicited mitochondrial hyperpolarization, which was accompanied by elevation of cytoplasmic and mitochondrial Ca^{2+} and increased NO production. These findings were consistent with previous data on H_2O_2- and ROI-induced IP3 production and Ca^{2+} release from endoplasmic reticulum and mitochondrial Ca^{2+} stores. Treatment of PBL with NO donor NOC-18 alone also elicited ROI production and Ca^{2+} release, indicating a positive-feedback regulation between NO and ROI signaling. Western blot analysis showed enhanced expression of eNOS and nNOS in CD3/CD28- or H_2O_2-stimulated PBL. This can be related to previous studies showing that both transcription rate and half-life of eNOS mRNA are enhanced by H_2O_2.

Activity of both eNOS and nNOS is turned on by elevation of Ca^{2+} and binding of Ca^{2+}/calmodulin. Expression of Ca-dependent NOS isoforms and absence of Ca-independent iNOS in PBL are consistent with involvement of CD3/CD28 costimulation-induced Ca^{2+} release in ensuing NO production. The present data are consistent with a key role for NO production in T cell activation-induced mitochondrial hyperpolarization, which, in turn, is regulated by Ca^{2+} and ROI at multiple levels.

Histamine modulates the cytokine production of immunocompetent cells, including T lymphocytes, by binding to histamine receptors on their cell surface. T cells express both type 1 and type 2 histamine receptors, histamine inhibits the production of Th1 cytokines such as IL-2 and interferon-γ (IFN) and enhances the secretion of Th2 cytokines such as IL-4, IL-5, IL-10 and IL-13. Histamine shifts the Th1/Th2 balance towards Th2, via regulation of JAK-STAT signal transduction pathway. Decreased production of INF-γ following histamine treatment, was reported in activated human blood mononuclear cells. Our present data confirm and extend the observations of others, regarding the immunoregulatory role of histamine. In the present study we show for the first time that T lymphocytes in vivo, in the absence of histamine, exhibit Th1-type cytokine dominance, as they produce higher levels of IFN-γ both at mRNA and protein levels. Our data indicate that histamine deficiency is associated with a markedly increased T cell NO production, and histamine directly regulates NO production. Thereafter NO may contribute to the shifted cytokine profile of

HDC-KO T cells. According to our data, mouse T cells predominantly express the neuronal (nNOS) isoform, while lower level of inducible (iNOS) and endothelial (eNOS) isoforms were detected. While the iNOS activity depends on transcription, the eNOS and the nNOS are constitutively expressed and are activated upon elevated intracellular Ca^{2+}. Cytoplasmic Ca^{2+} level is higher in HDC-KO T cells, which may activate eNOS and nNOS enzymes and induce NO production. According to our previous data, NO donor treatment increases both cytoplasmic Ca^{2+} concentration and T cell activation induced Ca^{2+} signal, which observation is in accordance with our present data. Furthermore the NO production and the T cell activation-induced NO signal are both higher in HDC-KO T cells. Our data suggest that histamine is an additional factor that regulates NO production, confirming an important role of NO in T cell activation.

Publications related to the theme

Koncz A, Pasztoi M, Mazan M, Fazakas F, Buzas E, Falus A, Nagy G. Nitric oxide mediates T cell cytokine production and signal transduction in histidine dexarboxylase knockout mice. ***J Immunol*** 2007 Nov. 15;179(10):6613-9. ***IF: 6,3***

Nagy G, **Koncz A**, Fernandez D, Perl A.Nitric oxide, mitochondrial hyperpolarization, and T cell activation. ***Free Radic Biol Med***. 2007 Jun 1; 42(11): 1625-31. ***IF:5,4***

Nagy G, **Koncz A**, Perl A Mitochondrial T cell signal transduction abnormalities in systemic lupus erythematosus ***Curr Immunol Rev*** 2005, 1, 62-68.

Perl A, Gergely P Jr, Nagy G, **Koncz A**, Banki K. Mitochondrial hypepolarization: a checkpoint of T-cell life, death and autoimmunoty. ***Trends Immunol*** 2004, 25, 360-7. ***IF:13,1***

Nagy G, **Koncz A**, Perl A. T cell activation induced mitochondrial hyperpolarization is mediated by Ca^{2+} and redox dependent production of nitric oxide. ***J Immunol*** 2003, 171, 5188-97**.** ***IF: 6,7***

Cumulative impact factor: 31,5:

Publications not related to the theme

Nagy G, Ward J, Mosser DD, **Koncz A**, Gergely P Jr, Stancato C, Qian Y, Fernandez D, Niland B, Grossman CE, Telarico T, Banki K, Perl A.Regulation of CD4 expression via recycling by HRES-1/RAB4 controls susceptibility to HIV infection. ***J Biol Chem***. 2006 Nov 10; 281(45):34574-91 ***IF: 5,8***

Gergely P Jr, Pullmann R, Stancato C, Otvos L Jr, **Koncz A**, Blazsek A, Poor G, Brown KE, Phillips PE, Perl A. Increased prevalence of transfusion-transmitted virus and cross-reactivity with immunodominant epitopes of the HRES-1/p28 endogenous retroviral autoantigen in patients with systemic lupus erythematosus.***Clin Immunol***. 2005 Aug;116(2):124-34. ***IF:3,2***

Nagy G, **Koncz A**, Perl A. T- and B-cell abnormalities in systemic lupus erythematosus. ***Crit Rev Immunol***. 2005, 25(2),123-40. ***IF: 3,2***

Nagy G, Clark J M, Buzas E, Gorman C, Pasztoi M, **Koncz A**, Falus A and Cope A P. Nirtic oxide production of T lymphocytes is increased in rheumatoid arthritis. ***Immunology Letters*** 2008 in press. ***IF: 2,3***

Koncz A, Nagy G, Perl A. Nitrogén monoxid függő mitokondrium bioszintézis systemas lupus erythematosusban ***Klinikai és kísérletes laboratóriumi medicina*** 2005. szeptember

Nagy G, Géher P, **Koncz A**, Perl As. Jelátviteli defektusok systemás lupus erythematosusban. ***Orvosi Hetilap***. 2005 Jul 31.

Cumulative impact factor: 46

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