Karolinska Institutet

Institutionen för medicin, Huddinge

Strategies for Modulation of Dendritic Cell Responses

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras på det engelska språket i föreläsningssalen 4V, Alfred Nobels Allé 8, Karolinska Universitetssjukhused Huddinge

Fredagen den 1 juni, 2012, kl 09:15

av

Annette E. Sköld

Huvudhandledare:
Docent Anna-Lena Spetz
Karolinska Institutet
Institutionen för medicin, Huddinge

Bihandledare:
Dr Lilian Walther Jallow
Apotekarsocieteten

Dr Barbro Mäkitalo
Smittskyddsinstitutet
Avdelningen för analys och prevention
Enheten för antibiotika och vårdhygien

Fakultetsopponent:
Professor Jolanda M. De Vries
Radboud University Nijmegen Medical Centre
Department of Tumor Immunology
Nijmegen Centre for Molecular Life Sciences

Betygsnämnd:
Professor Anders Vahlne
Karolinska Institutet
Institutionen för Laboratoriemedicin

Professor Petter Höglund
Karolinska Institutet
Institutionen för medicin, Huddinge

Docent Marie Larsson
Linköpings Universitet
Institutionen för klinisk och experimentell medicin

Stockholm 2012

ABSTRACT

With increased knowledge in dendritic cell (DC) biology, innate immune receptors and their ligands, and the shaping of adaptive responses, refined approaches to modulate our immune system are today emerging as treatment strategies for chronic infections and severe cancers. At the center of attention stand DCs – the innate immune cells that orchestrate the adaptive immune responses. In this thesis, strategies to activate and to inhibit DC activation are described, and the effect of different types of activation of DCs on HIV-1 infection is also investigated.

In **paper I**, we have characterized a novel strategy of TLR3 inhibition in DCs and in other TLR3 expressing cells. The TLR3 ligand poly I:C normally activates DCs to upregulate maturation markers CD80 and CD86 and to secreted pro-inflammatory cytokines. We found that simultaneous addition of oligodeoxynucleotides (ODNs) based on a phosphorothioate (PS) backbone together with poly I:C inhibited the TLR3-mediated DC activation. This inhibition was dependent on the structure of the ODN backbone, since ODNs built on a phosphodiester backbone did not have inhibitory effects, but independent of the sequence, since both CpG and non-CpG containing PS-ODNs had the ability to inhibit the effect of poly I:C. We could repeat the PS-ODN-mediated inhibition on poly I:C activation in three additional non-hematopoietic cell types. Upon investigation of the mechanism behind this observation, we determined that PS-ODNs are preferably taken up into DCs over poly I:C, and are thereby inhibiting the ligand interaction with TLR3. To confirm this finding *in vivo*, we treated cynomolgus macaques intranasally with the ligands, either alone or in combination, and measured the secreted cytokine levels. Significantly reduced levels of IL-12p40 were detected in animals receiving PS-ODNs compared to animals treated with poly I:C alone, and a similar trend was observed also for additional pro-inflammatory cytokines and chemokines measured. Hence, these findings encourage the development of PS-ODNs as a treatment strategy during TLR3-mediated pathology.

Our group has previously reported that irradiated activated PBMCs have the ability to induce DC maturation. In **paper II**, we set out to determine the underlying mechanism for this finding. First, we investigated whether the activated apoptotic cells (ACs) had to be phagocytosed for mediating their effect, but cell-cell contact was shown to be enough for DC maturation when co-cultured with ACs. We then tested if both cellular and supernatant fractions of activated ACs had the ability to mature DCs. Activated ACs were previously shown to release low levels of TNF-α, and we could confirm that the cytokine was a maturing agent in the supernatant fraction. The cellular fraction also matured DCs, and to investigate what molecules could be involved, we neutralized receptors previously shown to be stimulated by endogenous substances. We found that DC-SIGN, TLR4, and β2-integrins all were involved in AC-induced DC maturation, and a plausible ligand for TLR4 was shown to be heat shock protein 60. When investigating the intracellular signaling pathways mediating this effect, we determined that activated ACs induced signaling via Src family of tyrosine kinases, PI3K/Akt, JNK, and p38, and activated the NF-κB and AP-1 transcription factors.

We further investigated the effect of activated apoptotic T cells on DC and HIV-1 infection in **paper III**. These activated ACs, either HIV-1 infected or uninfected, had the ability to mature DCs, and also to reduce HIV-1 infection in DCs. This reduction was partly due to TNF-α produced by stimulated DCs, but mainly due to the increased expression of the HIV-1 host restriction factor APOBEC3G in DCs. In **paper IV**, we continued to investigate the expression of APOBEC3 family members in DCs upon treatment with TNF-α or IFN-α. We could confirm previous reports on expression of APOBEC3A, F, and G in DCs, and we also concluded that TNF-α, despite induction of DC activation, did not induce expression of APOBEC3 molecules, but more probably stimulated additional host restriction factors in DCs.

ISBN 978-91-7457-771-6