

Granulocyte products act as alarmins to enhance innate and adaptive immunity

De Yang^{1,2} and Joost J. Oppenheim²

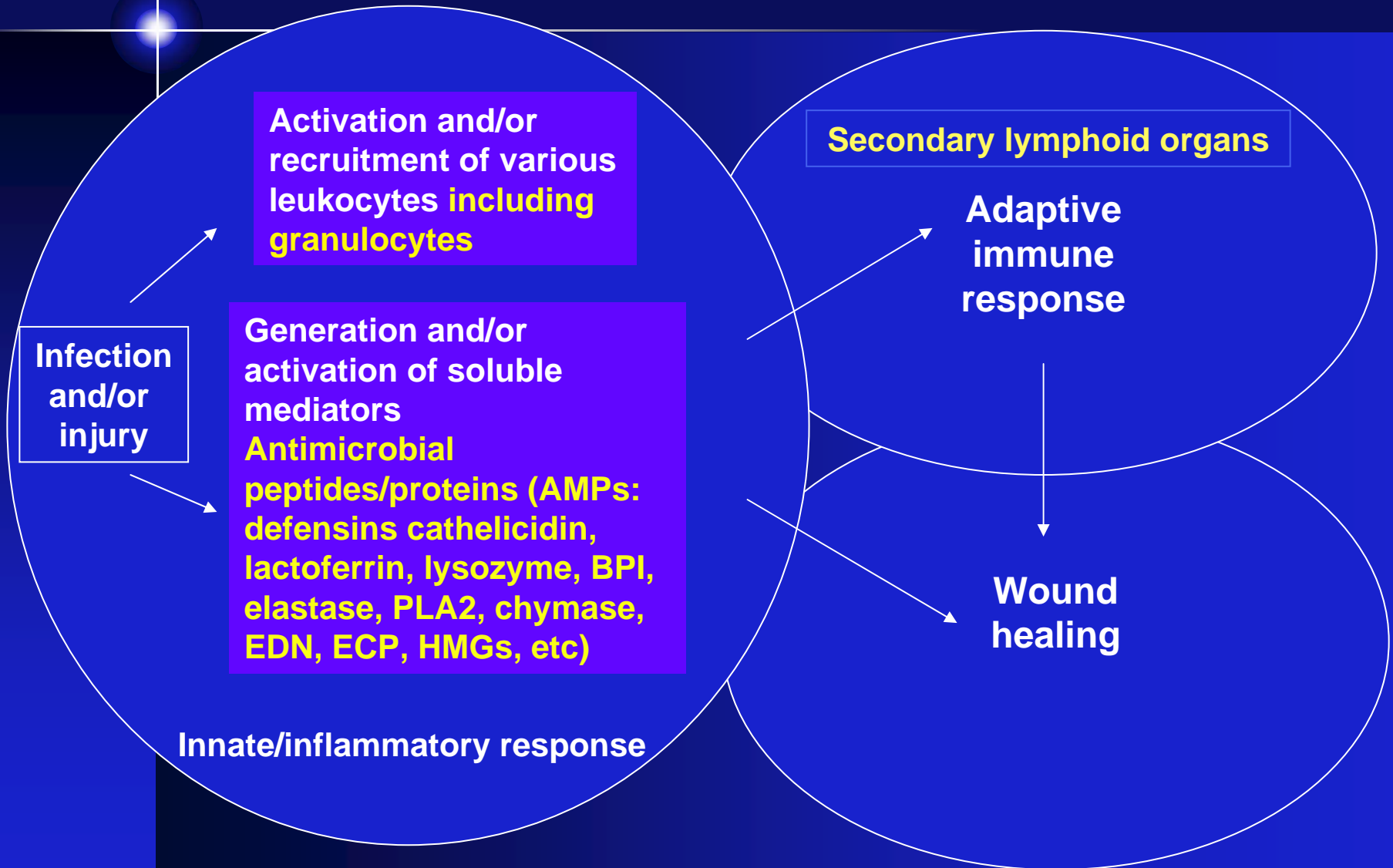
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Preview

1. From granulocyte products to alarmins
2. HMGN1 as a Th1-polarizing alarmin
3. Eosinophil-derived neurotoxin (EDN) as a Th2-polarizing alarmin
4. Conclusion

What are alarmins?

Granulocyte products in immunity



What are alarmins?

Identification of the effects of β -defensin to chemoattract and activate dendritic cells

β -defensins: linking innate and adaptive immunity through DC and T cell CCR6
(D Yang et al, *Science* 286:525-529; 1999)

1. Human β -defensin 2 (HBD2) is chemotactic for immature dendritic cells (iDC) and peripheral blood memory T cells.
2. β -defensin 2 utilizes CCR6 as the receptor for chemoattracting target cells.

Toll-like receptor 4-dependent activation of dendritic cells by β -defensin 2
(A Biragyn et al, *Science* 298:977-979; 2002)

1. Mouse β -defensin 2 induces full maturation of DCs.
2. TLR4 mediates the DC-activating effect of β -defensin 2 .
3. β -defensin 2-tumor antigen (of mouse B-cell lymphoma) fusion product enhances B-cell lymphoma-specific immune responses.

What are alarmins?

AMPs capable of acting as APC chemoattractant, activator, and immune enhancer

Mediator		Activity	APC chemoattraction		APC activation		Immune enhancement
			Target cells	Receptor	Target cells	Receptor	
Defensins		HNP1~4	DC, M ϕ	?	M ϕ	?	+
		HBD1-4	DC, M ϕ	CCR6, other?	DC, M ϕ	TLR2/1	+
		mBD2-3	DC	CCR6	DC	TLR4	+
		mBD14	DC, M ϕ	CCR6, other?	n.t.		n.t.
		mBD29	Pre-DC	CCR6	n.t.		n.t.
Cathelicidins		LL-37	Mo, M ϕ	FPRL1	DC, M ϕ	P2X7	+
		CRAMP	Mo, M ϕ	FPR2			
EARs		EDN	DC	?	DC	?	+
		mEAR2	DC	?			
Iron-binder		Lactoferrin	Mo, DC	?	Mo, M ϕ	?	+
Cathepsin		Cathepsin G	Mo	FPR	n.t.		n.t.
HMGs		HMGB1	DC	RAGE, GPCR	DC, Mo, M ϕ	RAGE, TLR	+
Saposin-like		Granulysin	DC	?	DC, M ϕ	?	n.t.

Note: 1. Lysozyme, elafin, SLPI, PLA2, and BPI do not have the aforementioned activities.
2. N.t. = not tested.

What are alarmins?

The proposal of alarmin concept

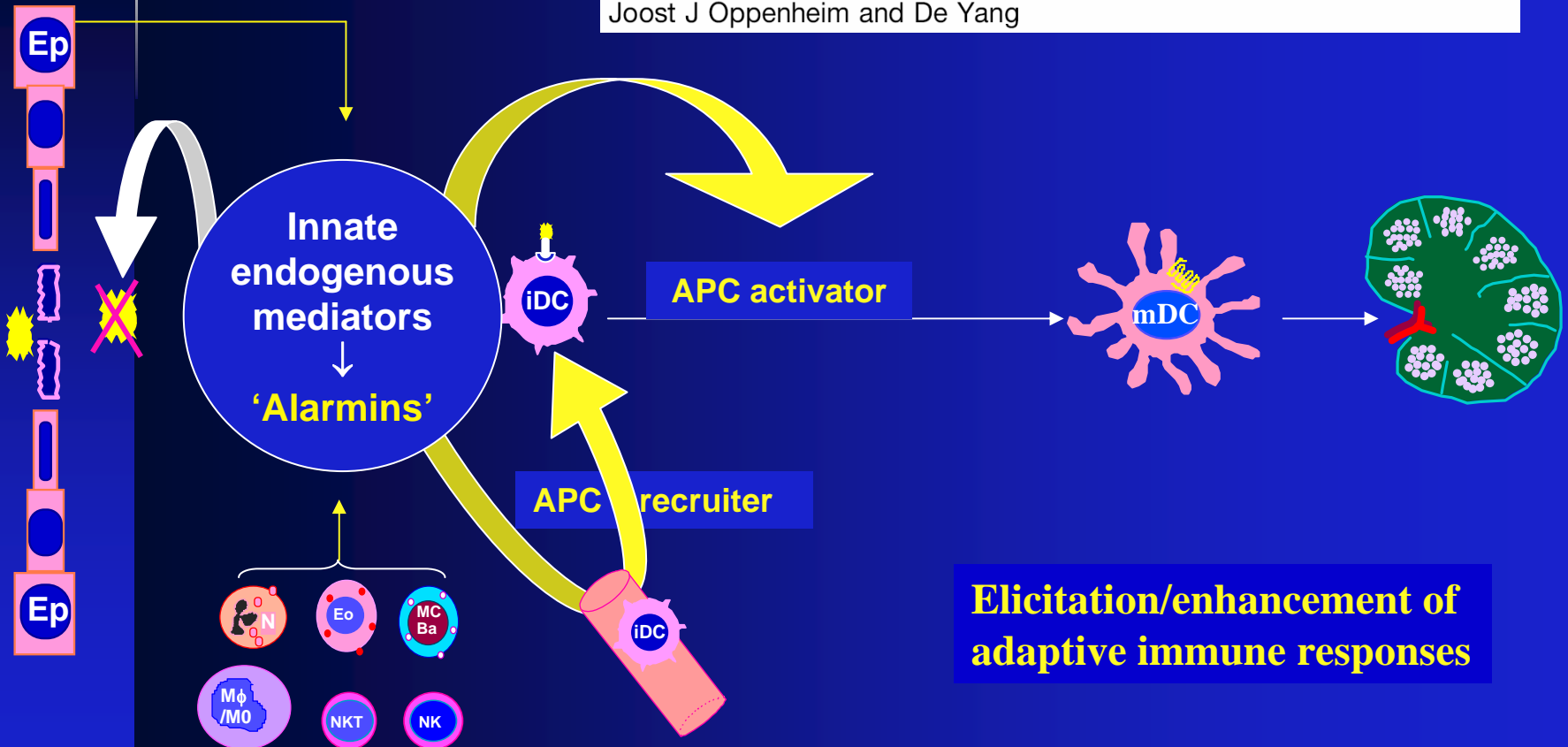


Cur Opin Immunol 2005, 17:359

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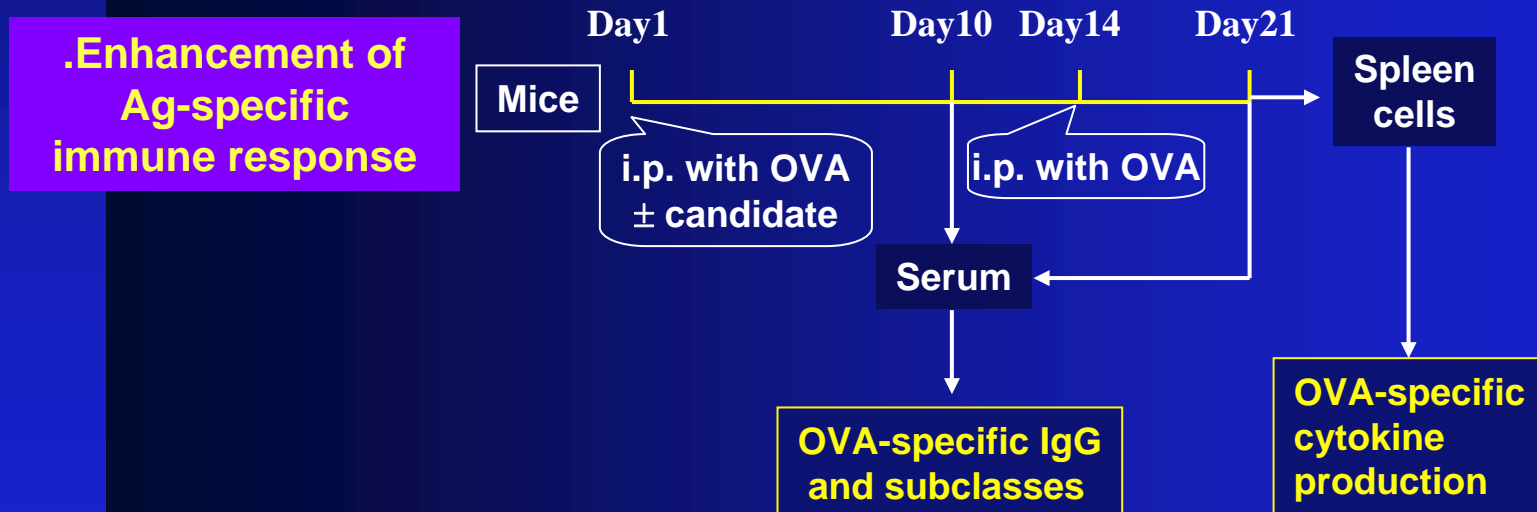
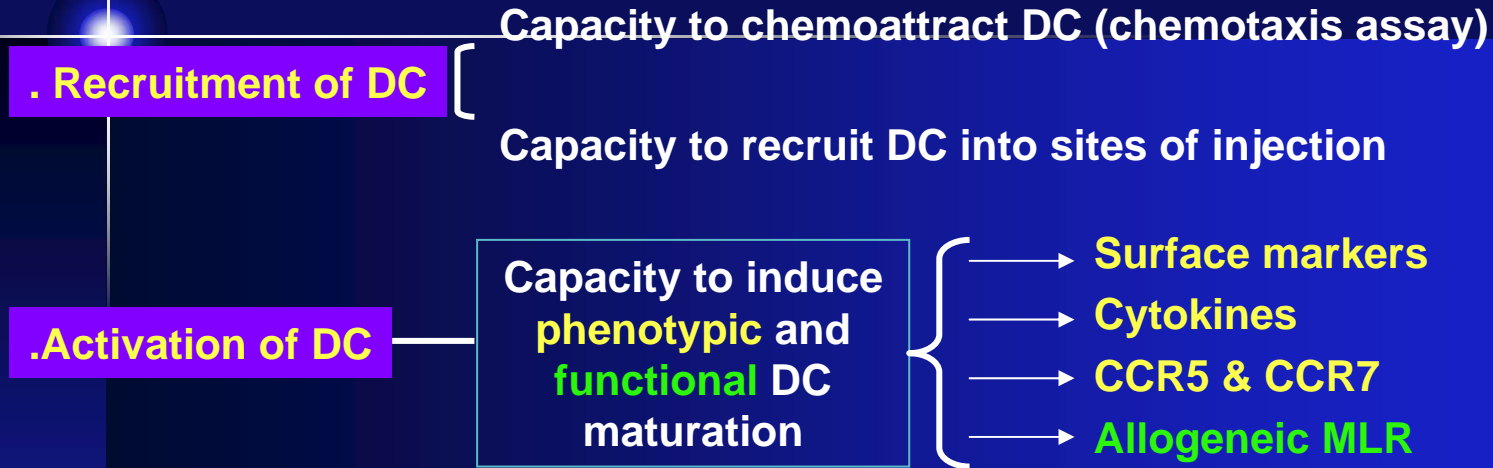
Alarmins: chemotactic activators of immune responses
Joost J Oppenheim and De Yang



Elicitation/enhancement of adaptive immune responses

What are alarmins?

How to determine if a given mediator is an alarmin or not?

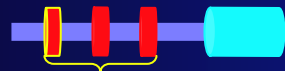


HMGN1 as a Th1-polarizing alarmin

Why to study HMGN1?

Classification

HMGA family



AT hooks

HMGA1a/b
HMGA1c*
HMGA2

HMGB family



Box1 Box2

HMGB1
HMGB2
HMGB3

HMGN family



NBD CHUD

HMGN1, HMGN2
HMGN3a/b, HMGN4
Nsbp1 (NBD-45)

Expression

Intranuclear

Abundant in embryonic tissues, differentially downregulated in adult tissues. Relative abundance: HMGBs>HMGNs>HMGAs.

Extracellular

?

HMGB1 can be secreted by Mφ

HMGNs can be released by PBMC

Function

Intranuclear

Regulation of development, differentiation, and cellular processes by binding to DNA and nucleosome and subsequent modulation of the transcription of various genes.

Extracellular

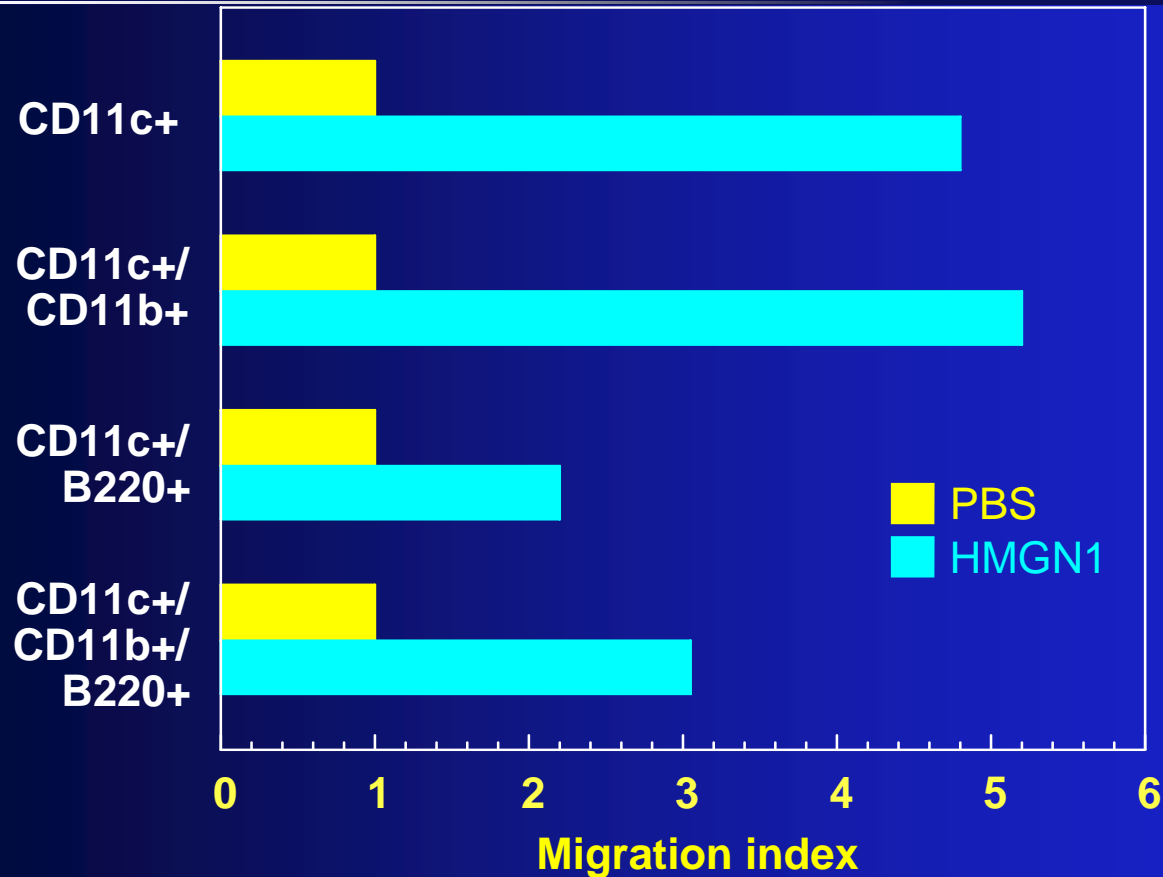
?

HMGB1 regulates cell migration, activation, and acts as an alarmin

Can HMGNs act as alarmins?

HMGN1 as a Th1-polarizing alarmin

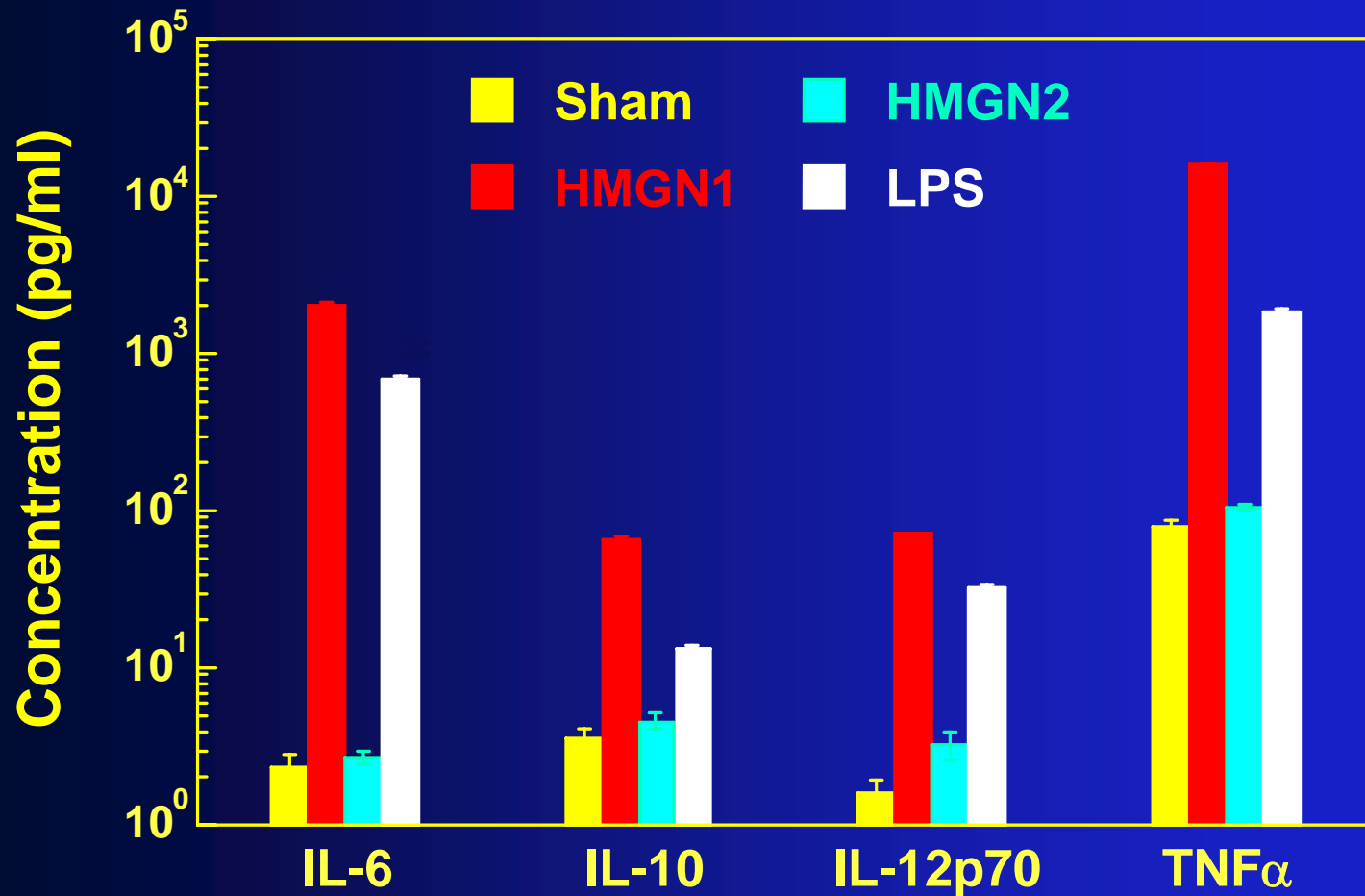
HMGN1 induces recruitment of DCs into mouse peritoneal cavity (4h)



$$\text{Migration index} = \frac{\text{Cell No. in the presence of HMGN1}}{\text{Cell No. in the absence of HMGN1 (PBS)}}$$

HMGN1 as a Th1-polarizing alarmin

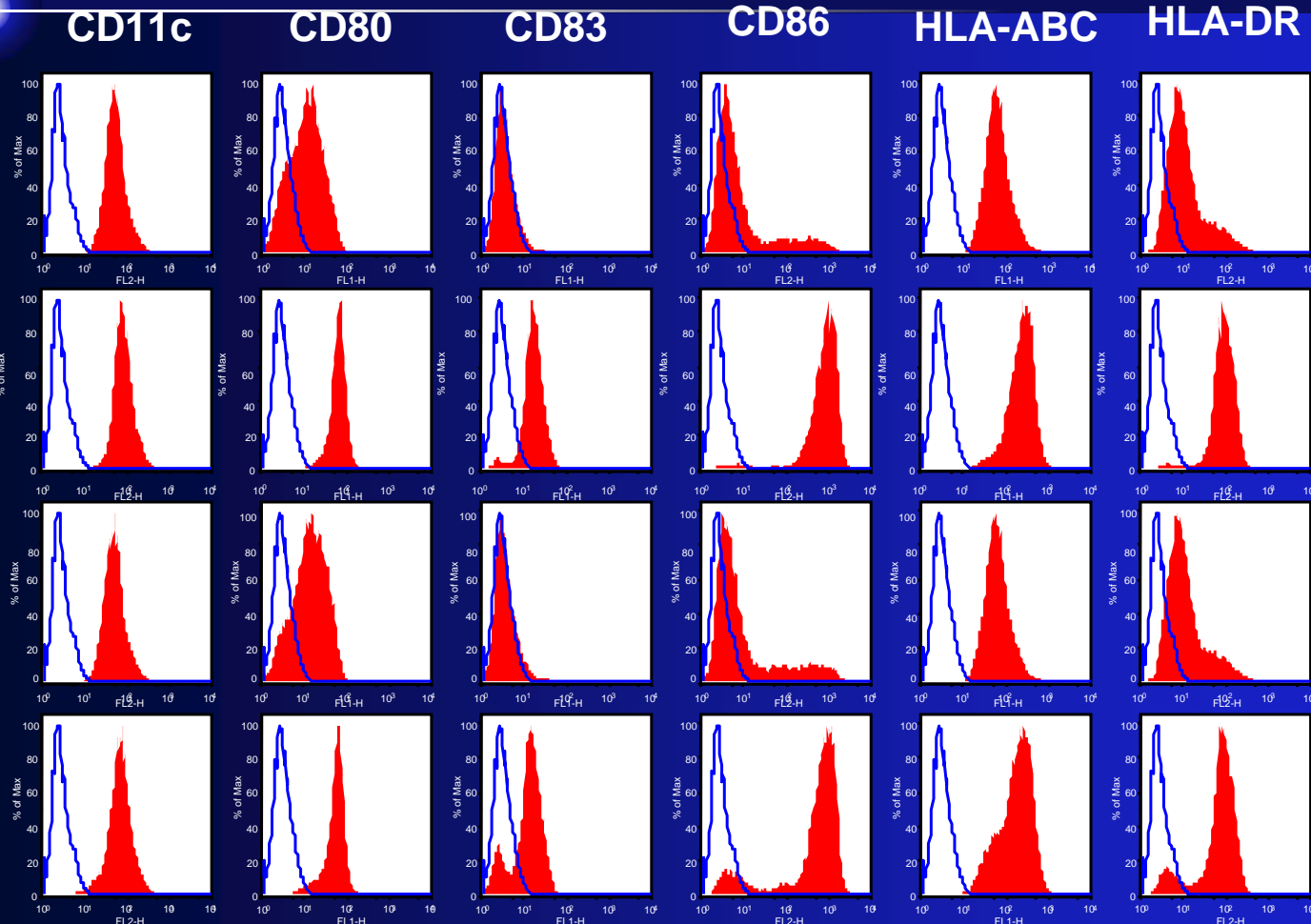
HMGN1 activation of DCs: 1. Cytokine induction



HMGN1 as a Th1-polarizing alarmin

HMGN1 activation of DCs: 2. Upregulation of surface markers

Cell number



Sham

HMGN1

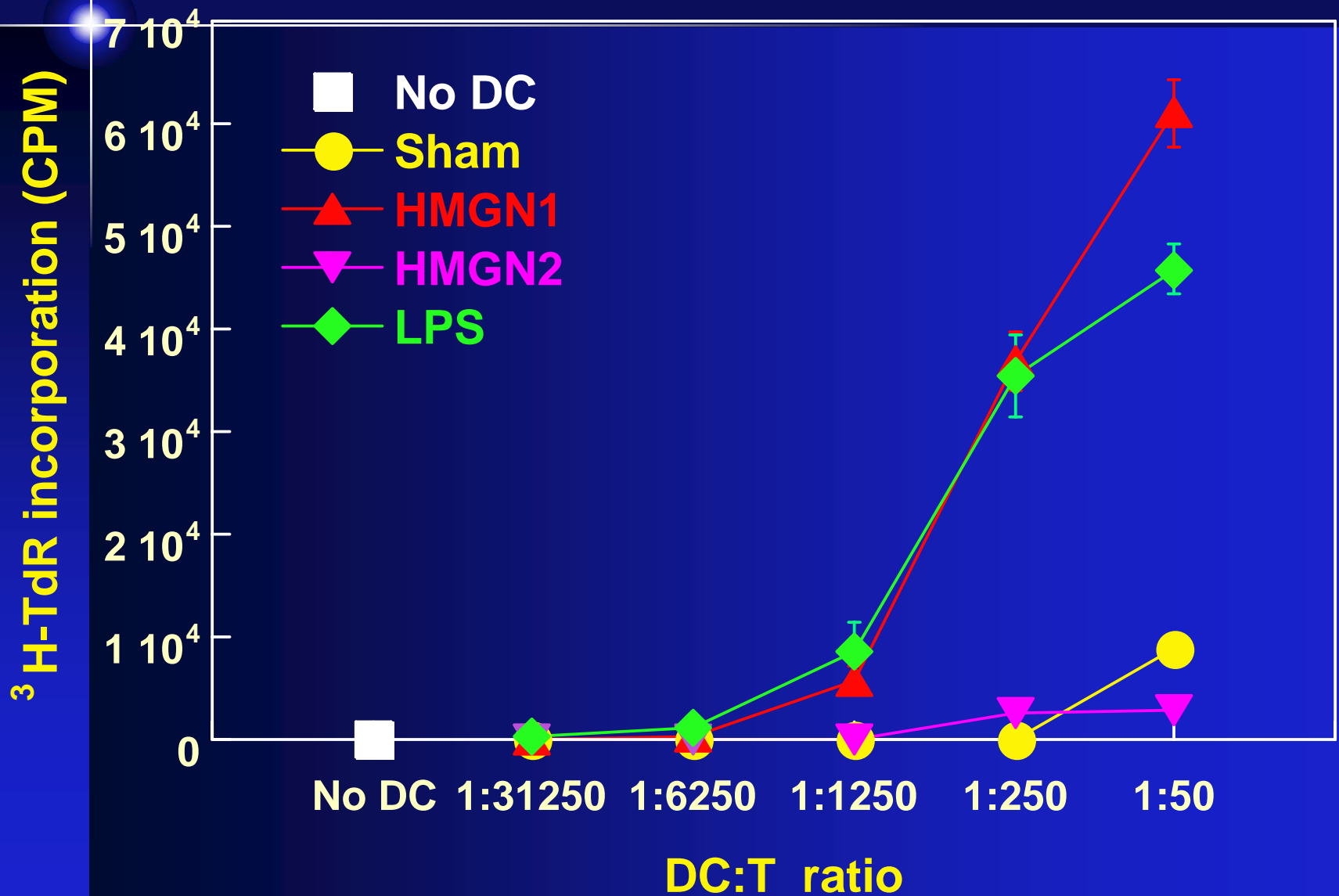
HMGN2

LPS

Relative fluorescence intensity (Log)

HMGN1 as a Th1-polarizing alarmin

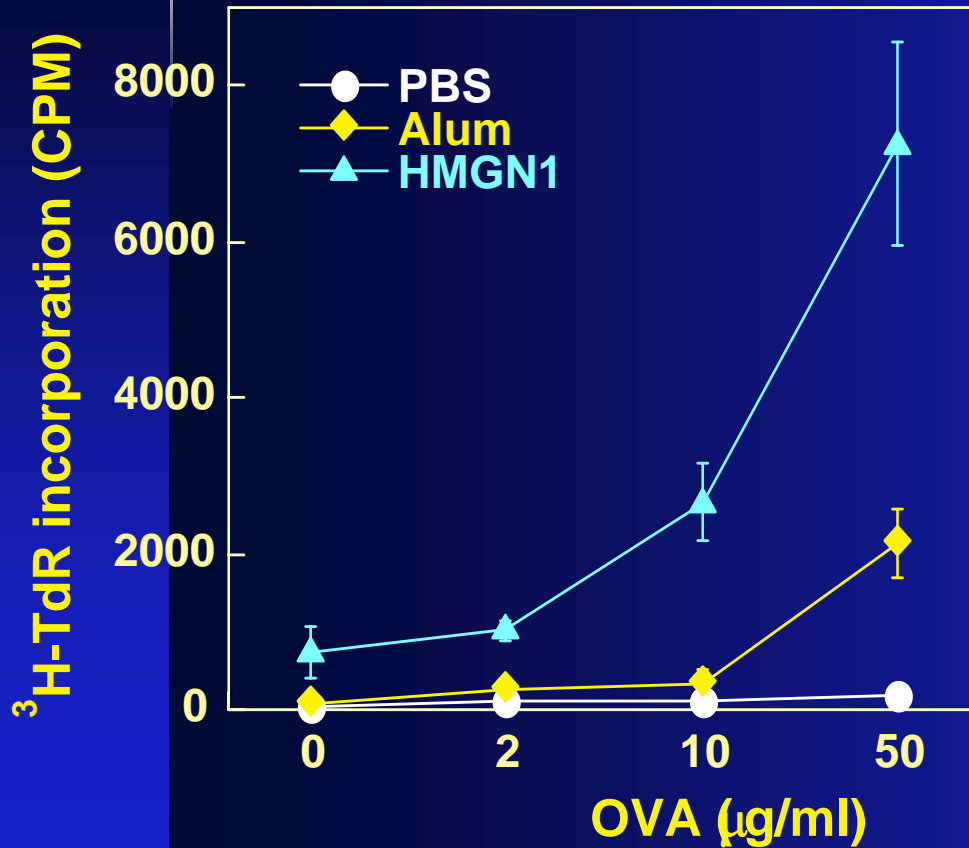
HMGN1 activation of DCs: 3. Functional maturation



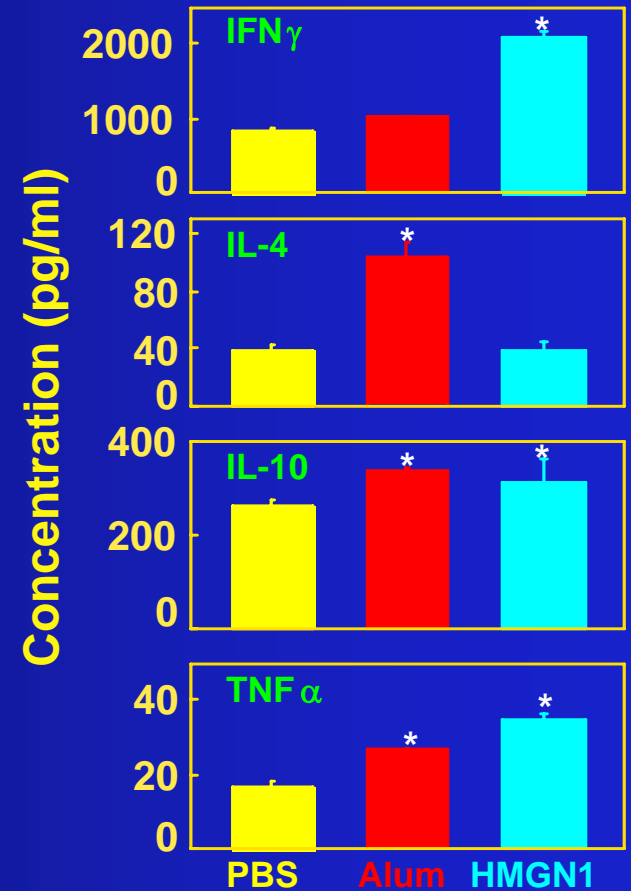
HMGN1 as a Th1-polarizing adjuvant

HMGN1 promotes OVA-specific Th1 immune response

OVA-specific
splenocyte
proliferation



OVA-specific
cytokine
production



HMGN1 as a Th1-polarizing alarmin

Summary

- **HMGN1 is capable of inducing DC activation as evidenced by induction of multiple cytokines, upregulation of surface molecules indicative of maturation, and enabling DCs to activate naïve T cells.**
- **HMGN1 can induce the recruitment of DCs.**
- **HMGN1 is able to promote OVA-specific immune response, favoring the polarization of T cells into Th1 (IFN γ -producing) direction.**
- **How HMGN1 attracts and/or activates is currently under investigation.**

Eosinophil-derived neurotoxin (EDN) as a Th2-polarizing alarmin

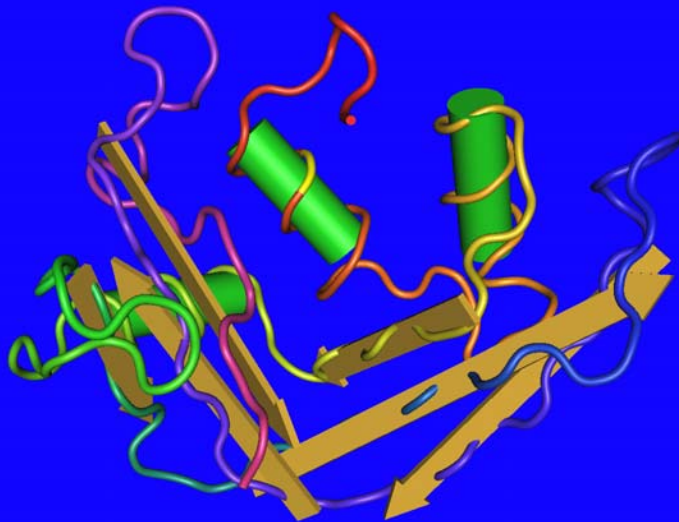
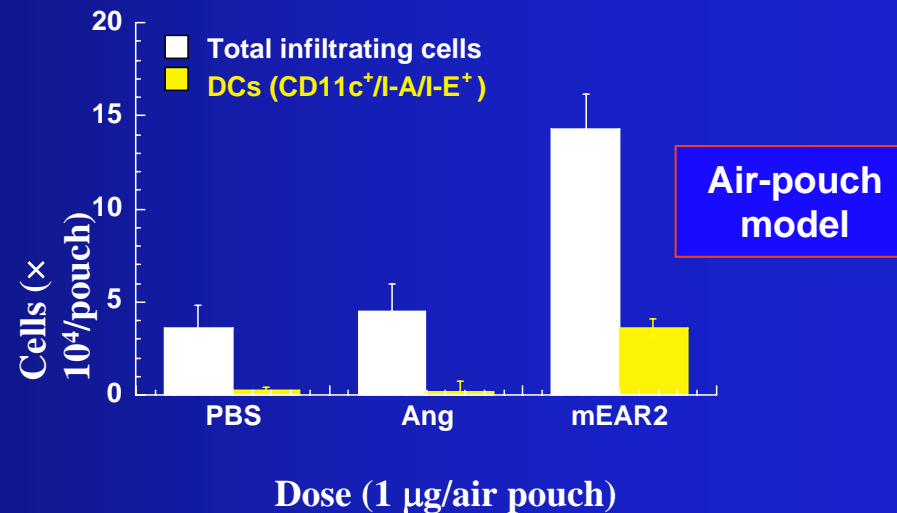
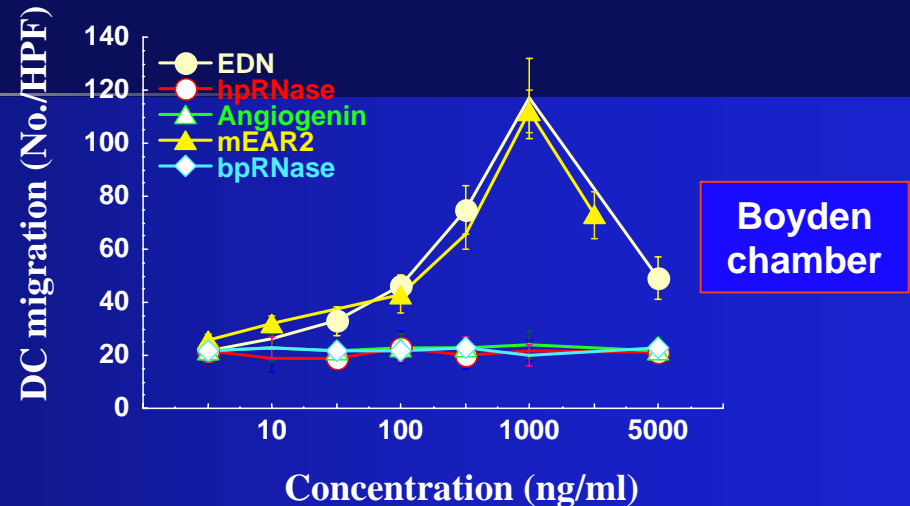
EDN is capable of inducing DC migration and recruitment

EDN: a 16-kDa cationic glycoprotein belonging to EAR superfamily.

Source: eosinophils, PMN, M ϕ , and certain epithelial cells.

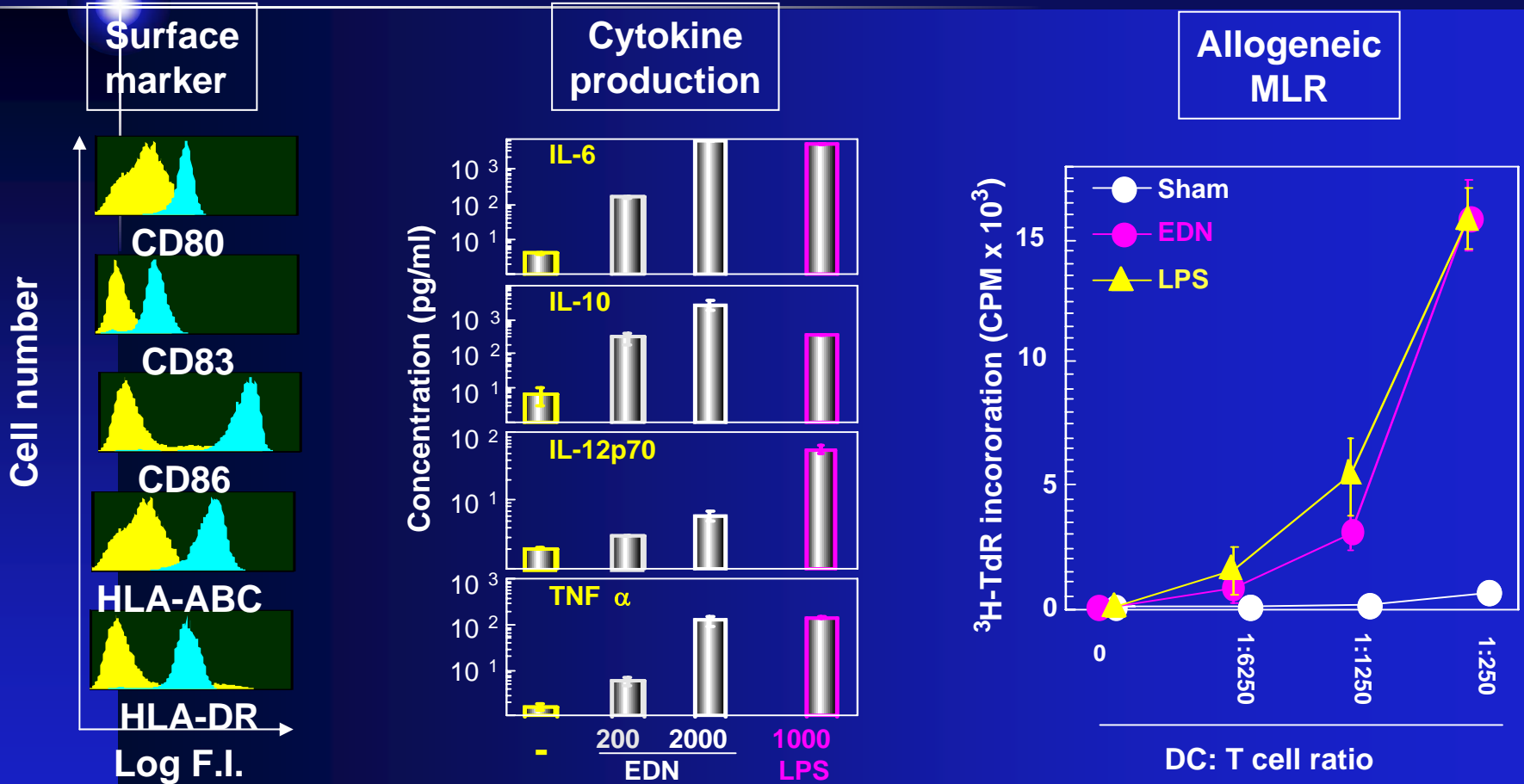
Structure: 136 a.a. folded into two lobes, each consisting of three anti-parallel β -sheets.

Activity: RNase; antimicrobial (helminth, viruses).



Eosinophil-derived neurotoxin (EDN) as a Th2-polarizing alarmin

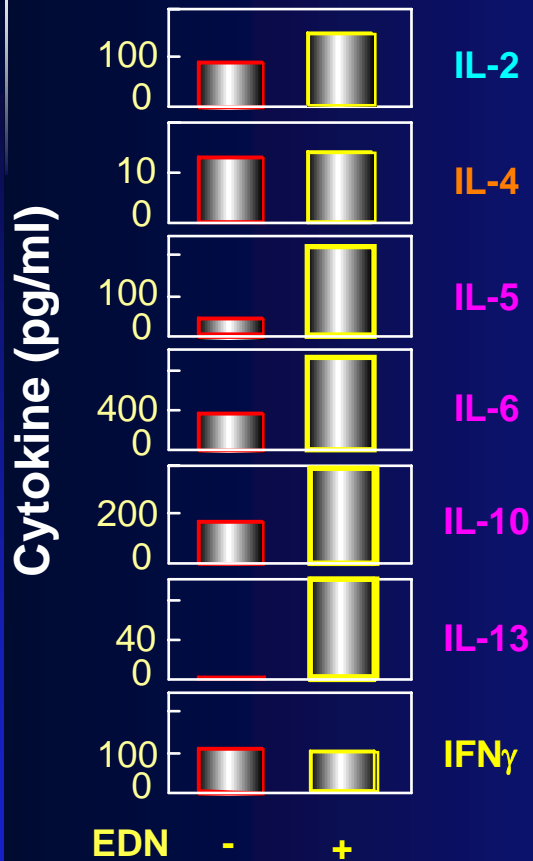
EDN induces maturation of human DCs



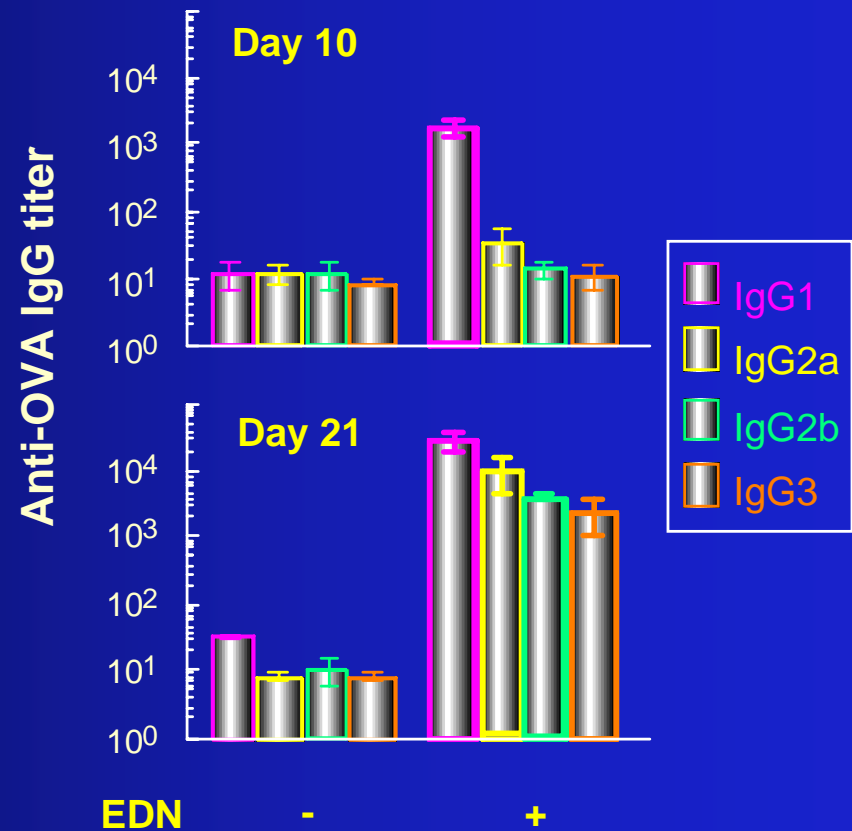
Eosinophil-derived neurotoxin (EDN) as a Th2-polarizing alarmin

EDN promotes OVA-specific Th2 response

OVA-specific cytokines



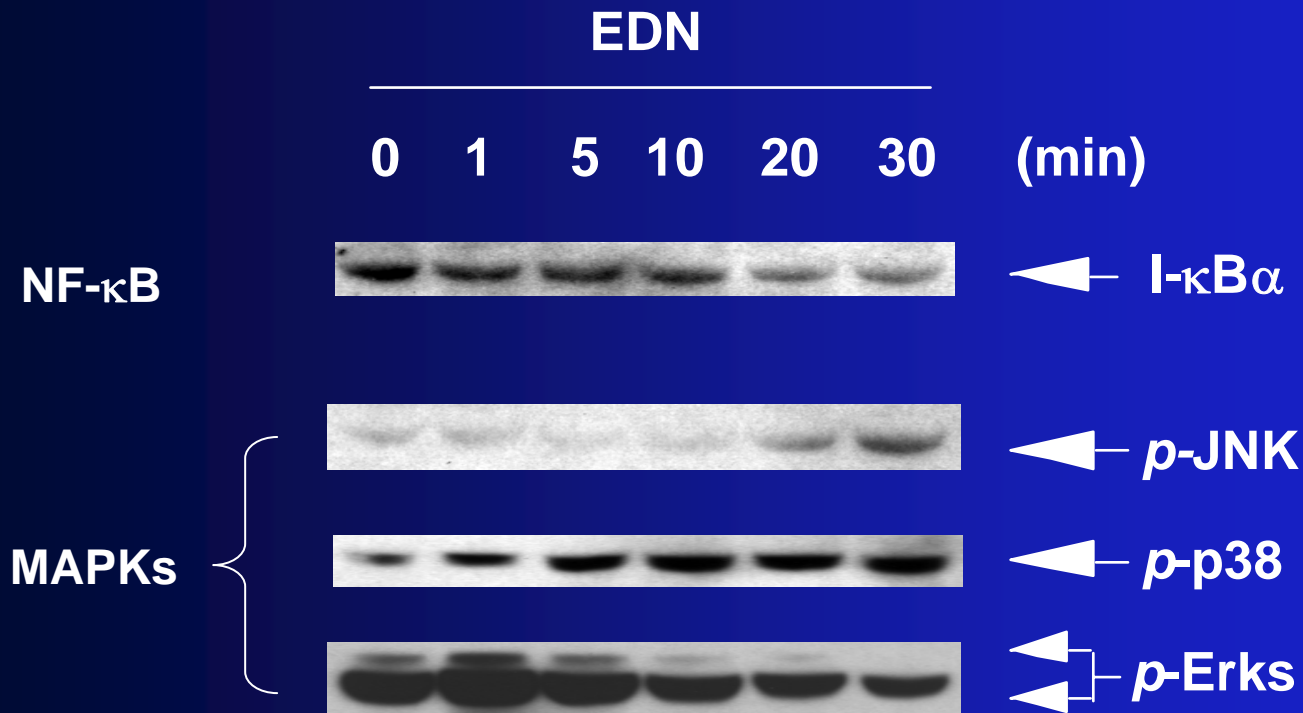
OVA-specific Abs



Eosinophil-derived neurotoxin (EDN) as a Th2-polarizing alarmin

How does EDN act?

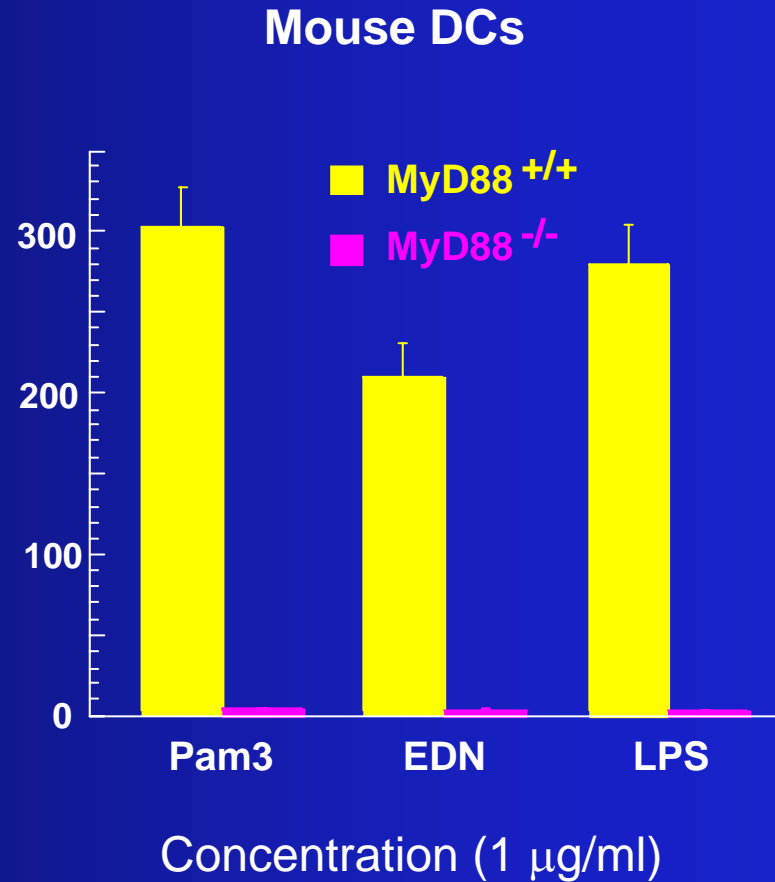
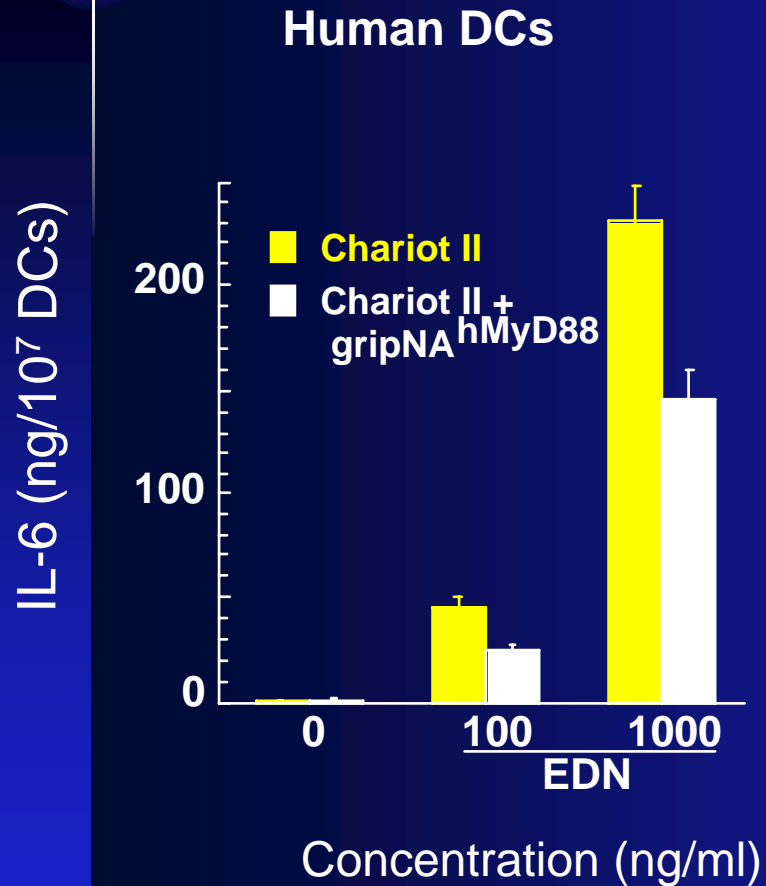
EDN activates NF- κ B and multiple MAPKs in DCs



Eosinophil-derived neurotoxin (EDN) as a Th2-polarizing alarmin

How does EDN act?

EDN induction of DC cytokines depends on MyD88

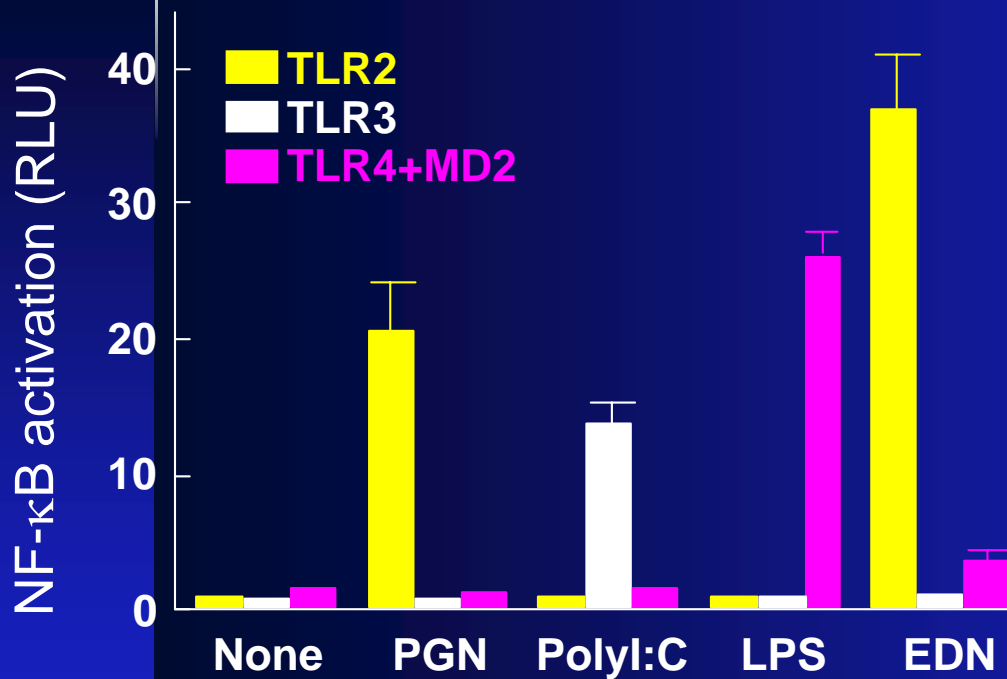


Eosinophil-derived neurotoxin (EDN) as a Th2-polarizing alarmin

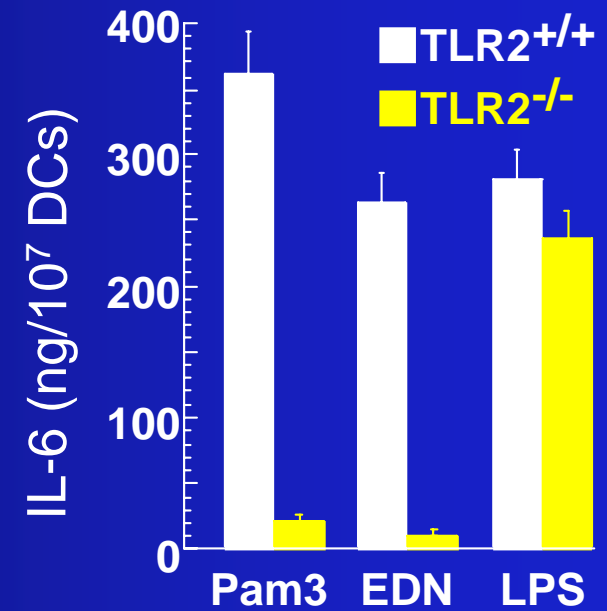
How does EDN act?

EDN triggers the activation of TLR2

NF- κ B activation in HEK293 cells



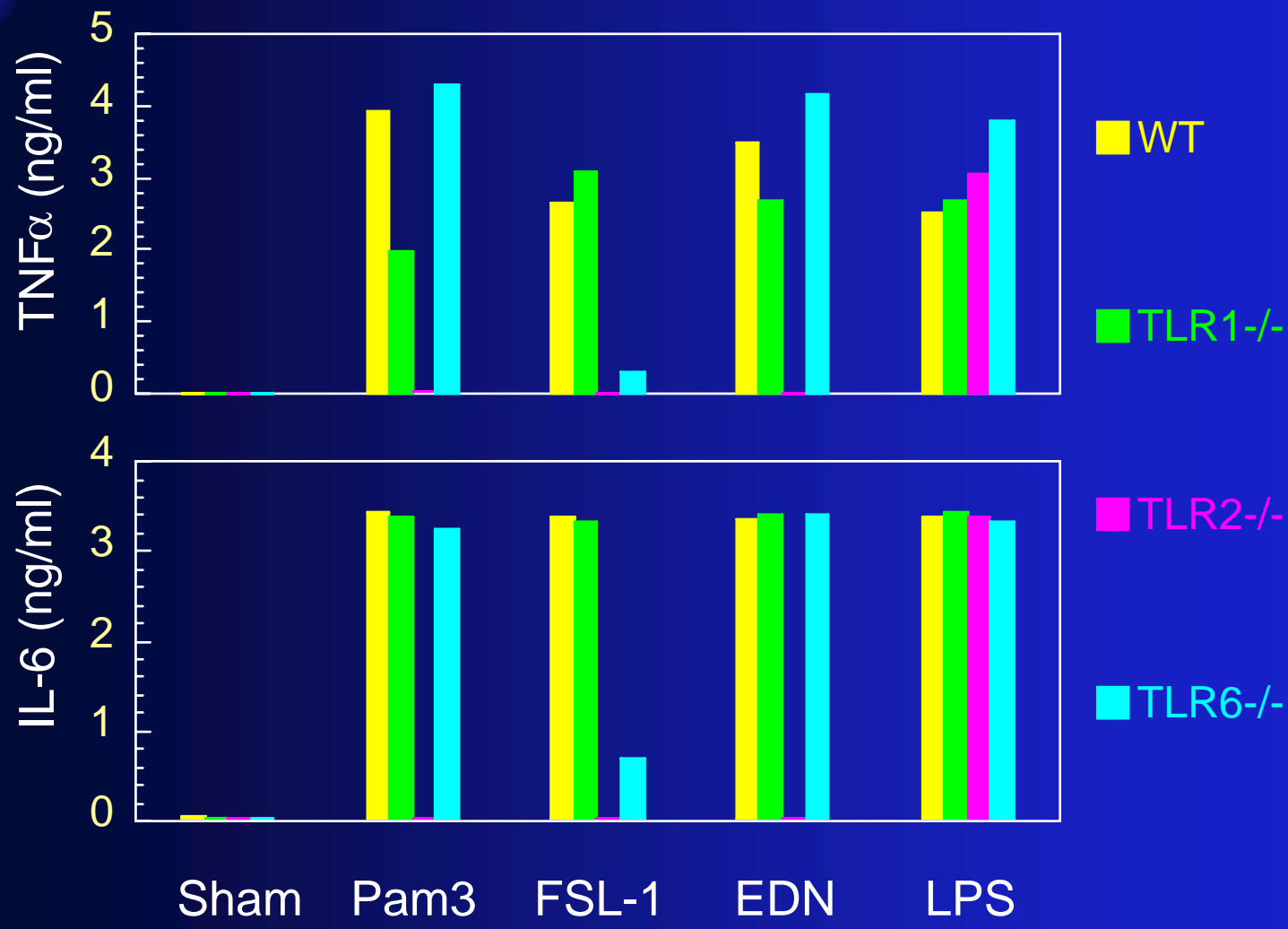
IL-6 production by mouse DC



Eosinophil-derived neurotoxin (EDN) as a Th2-polarizing alarmin

How does EDN act?

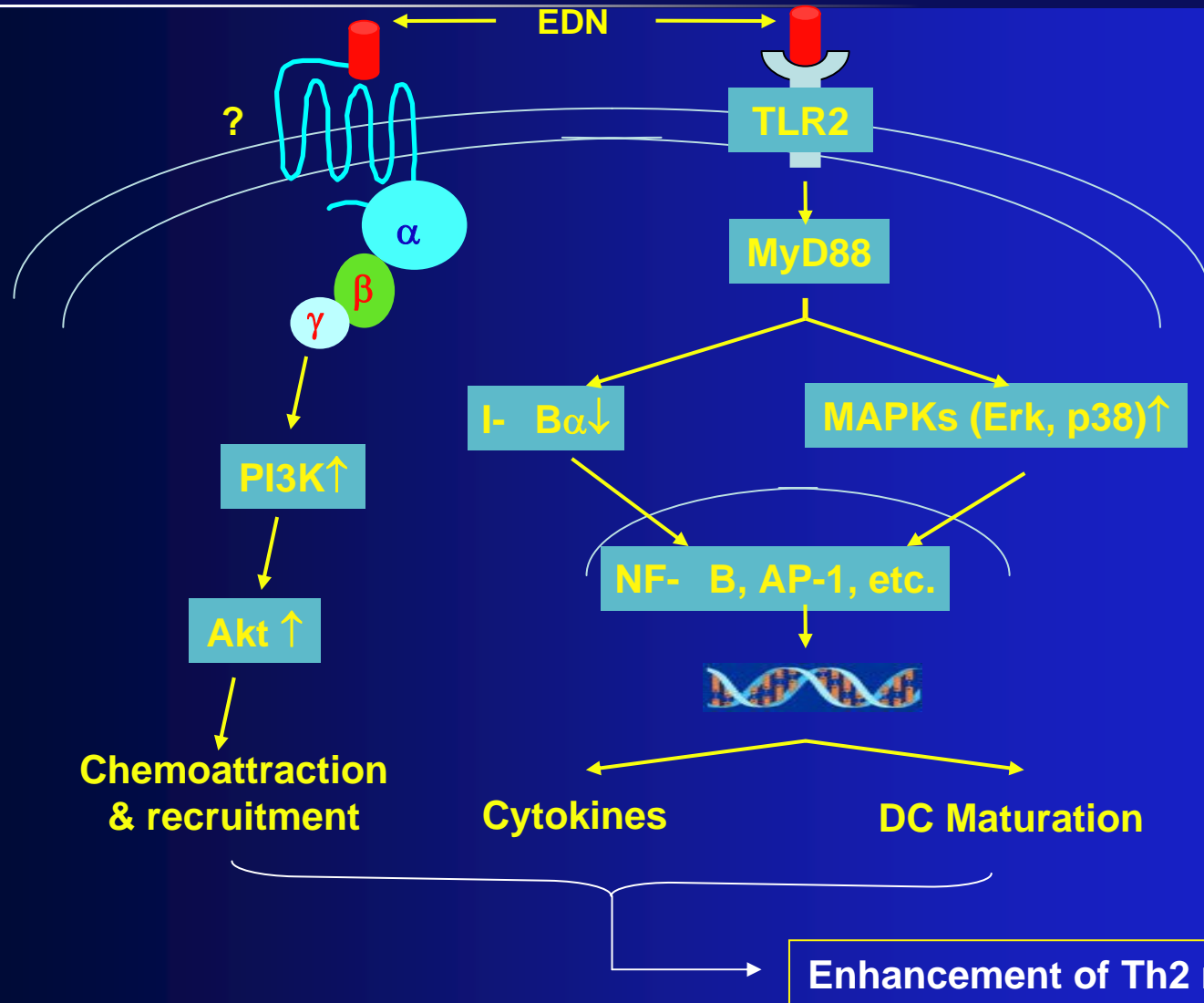
EDN activation of TLR2 is independent of TLR1 or TLR6



Eosinophil-derived neurotoxin (EDN) as a Th2-polarizing alarmin

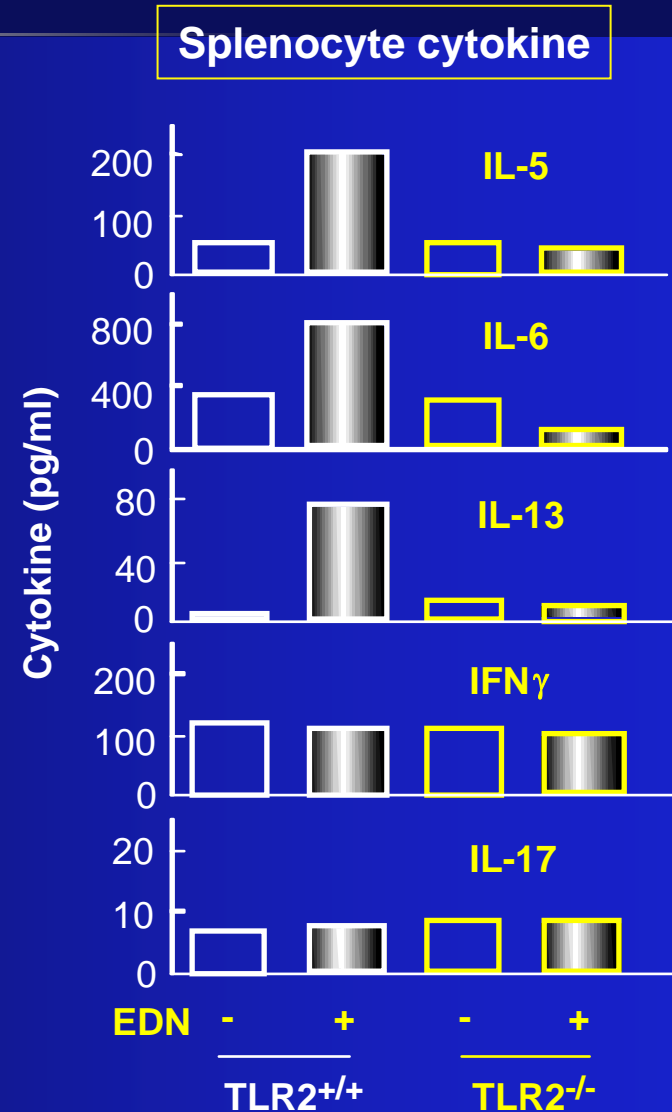
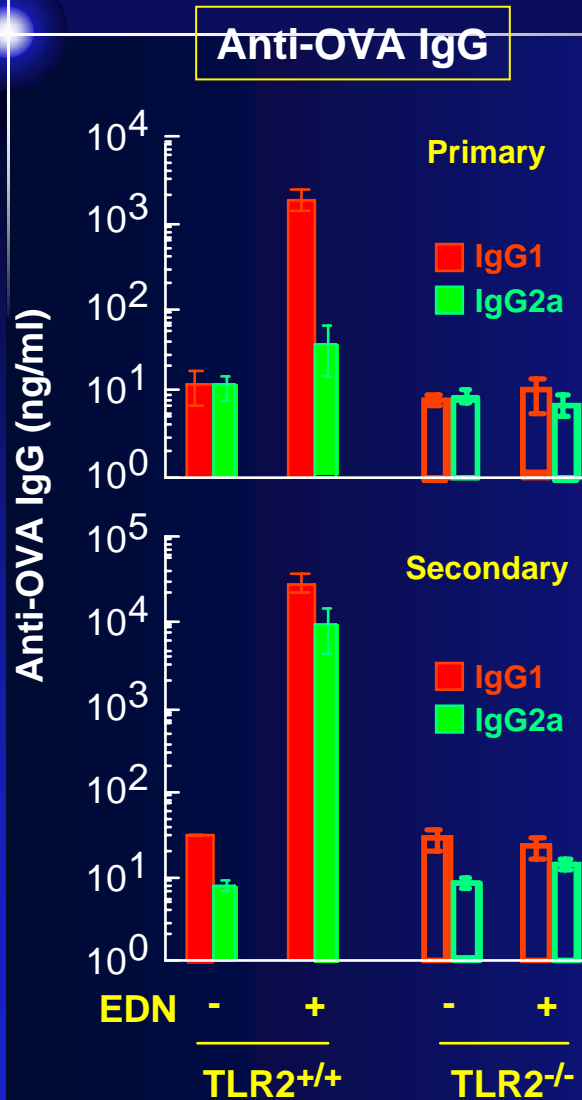
How does EDN act?

EDN induced signal transduction pathways



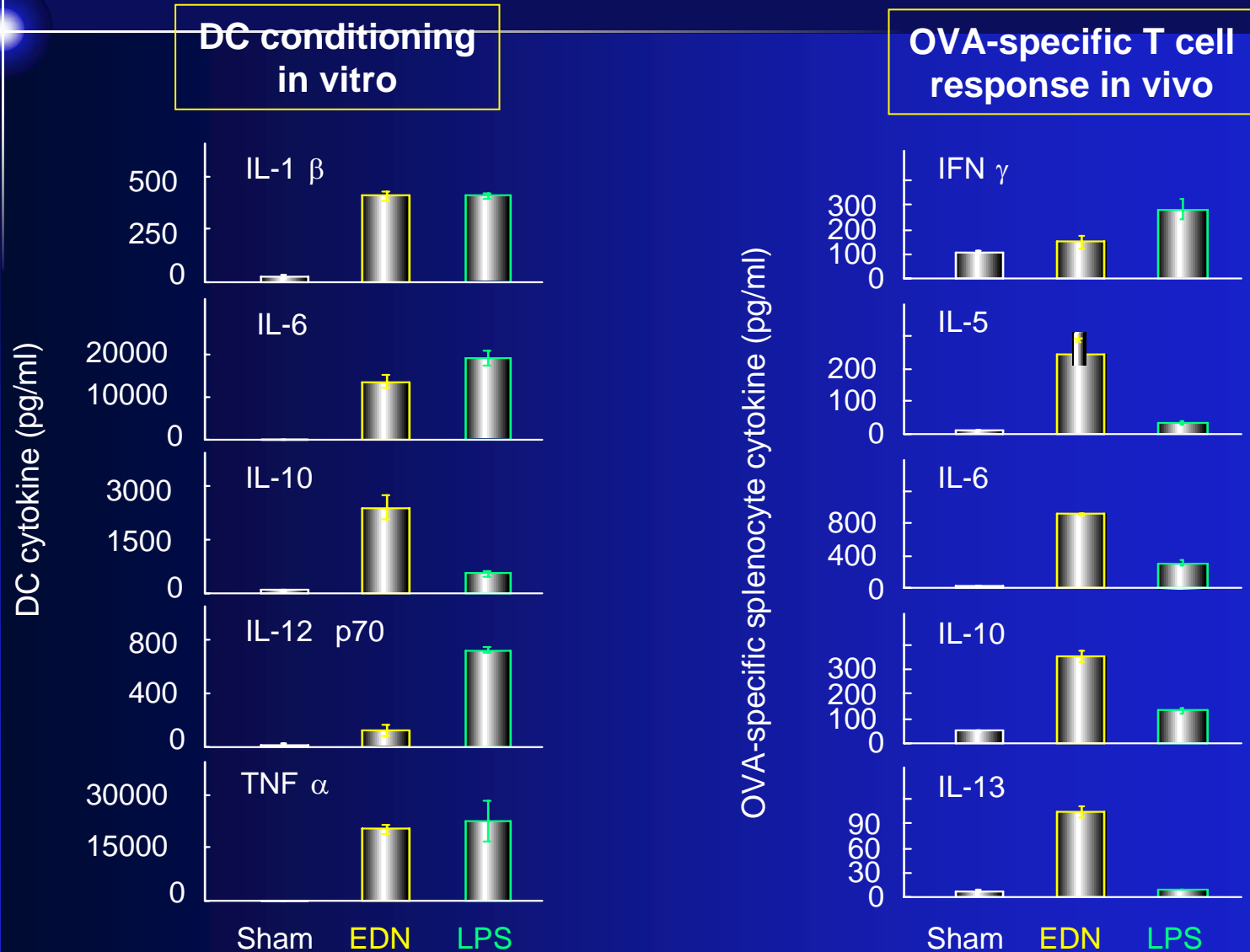
Eosinophil-derived neurotoxin (EDN) as a Th2-polarizing alarmin

EDN enhancement of anti-OVA Th2 response is mediated by TLR2



Eosinophil-derived neurotoxin (EDN) as a Th2-polarizing alarmin

OVA-loaded DCs treated with EDN in vitro promote Th2 response in vivo



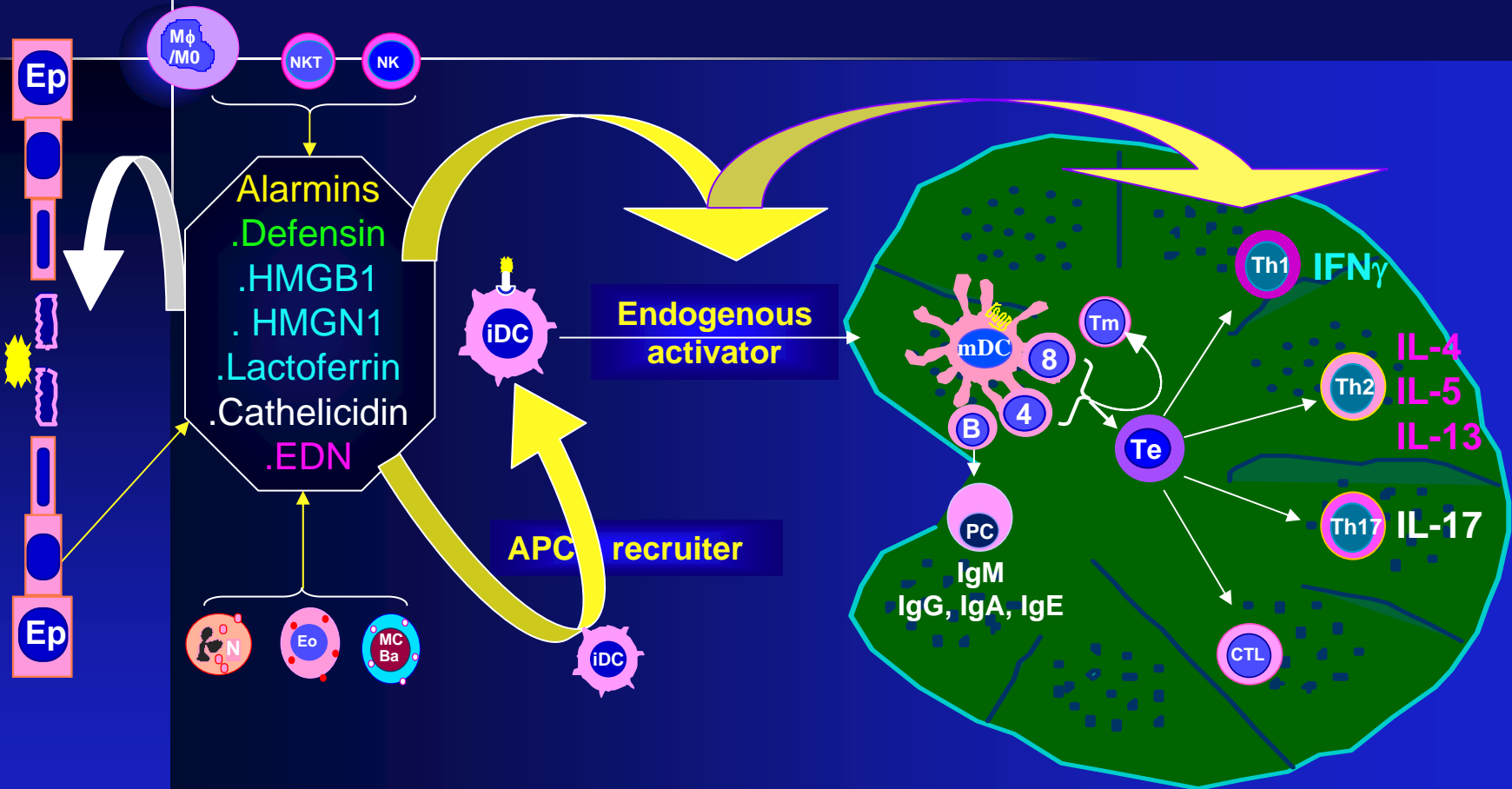
Eosinophil-derived neurotoxin (EDN) as a Th2-polarizing alarmin

Summary

- 1. EDN acts as an alarmin by inducing DC recruitment, activation, and promoting antigen-specific immune responses.**
- 2. EDN activation of DCs is mediated by TLR2.**
- 3. EDN selectively promotes Th2 immune response, in which both DCs and TLR2 are critical.**
- 4. EDN's effect on immune response may contribute to Th2 polarization associated with allergic and/or certain parasite infection.**

Conclusions and perspectives

Granulocyte products as alarmins to enhance and regulate innate and adaptive immunity



1. The mechanism(s) of alarmins' action (receptor and signaling pathway)
2. Can blockade of alarmins ameliorate inflammation?
3. The potential utilization of alarmins as adjuvants for vaccination or antitumor immunotherapeutic intervention?

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