

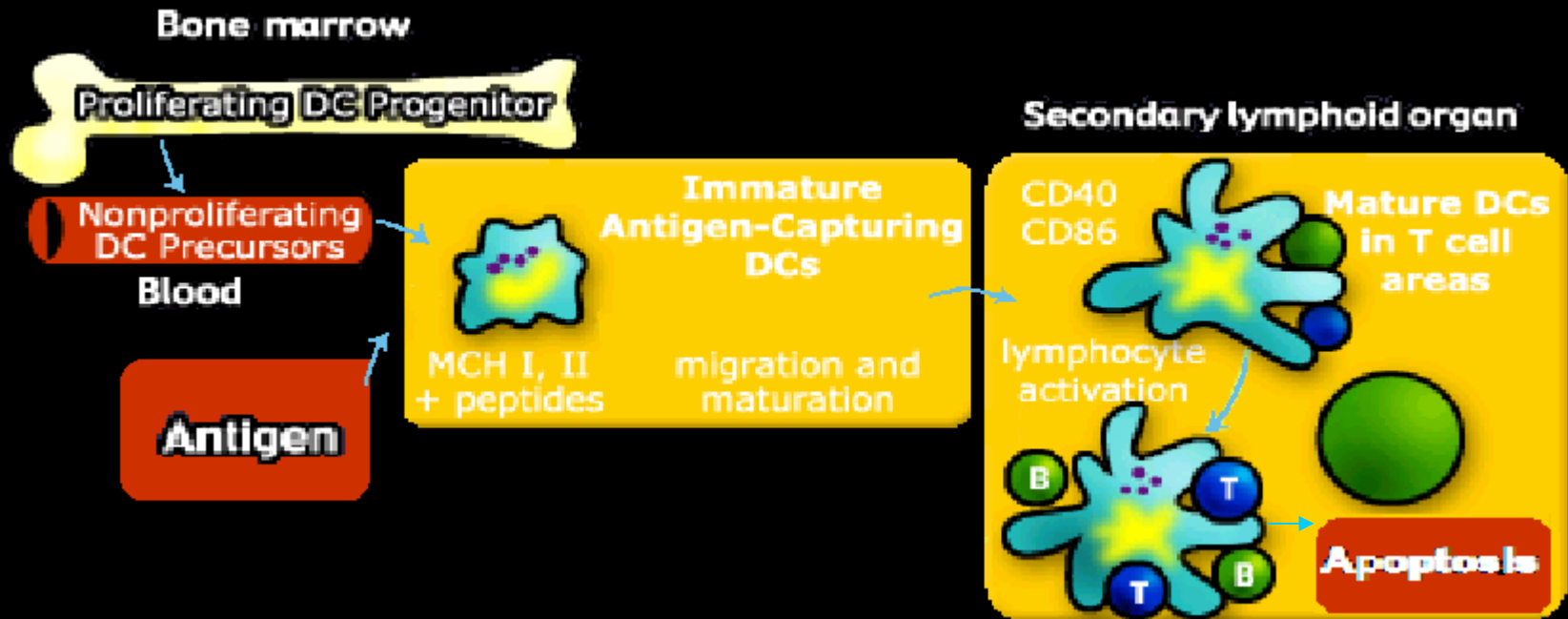
Effect of Btk Mutation on Toll-Like Receptor Signaling in Myeloid Dendritic Cells

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Dendritic cells

- antigen presenting cells
- stimulation of naive T cells
- life cycle of DC



- Toll-like receptors

Bruton's tyrosin kinase

- protein in B cells development
- mutation – lack of B cells and antibodies
 - Bruton's agammaglobulinaemia, X-linked agammaglobulinaemia
- Jefferies 2003 – btk in TLR signaling
(TLR4, TLR7, TLR8, TLR9)

Does the mutation in btk affect TLR signaling and DC function?

- all TLR with known ligands investigated

TLR2	Zymosan, LTA,
TLR6	Peptidoglycan
TLR3	Poly(I:C)
TLR4	Lipopolysaccharide
TLR5	Flagellin
TLR7	Loxoribine, ssRNA
TLR8	ssRNA
TLR9	CpG

DC generation

peripheral blood



Rosette Sep CD14 enrichment Coctail
Gradient centrifugation

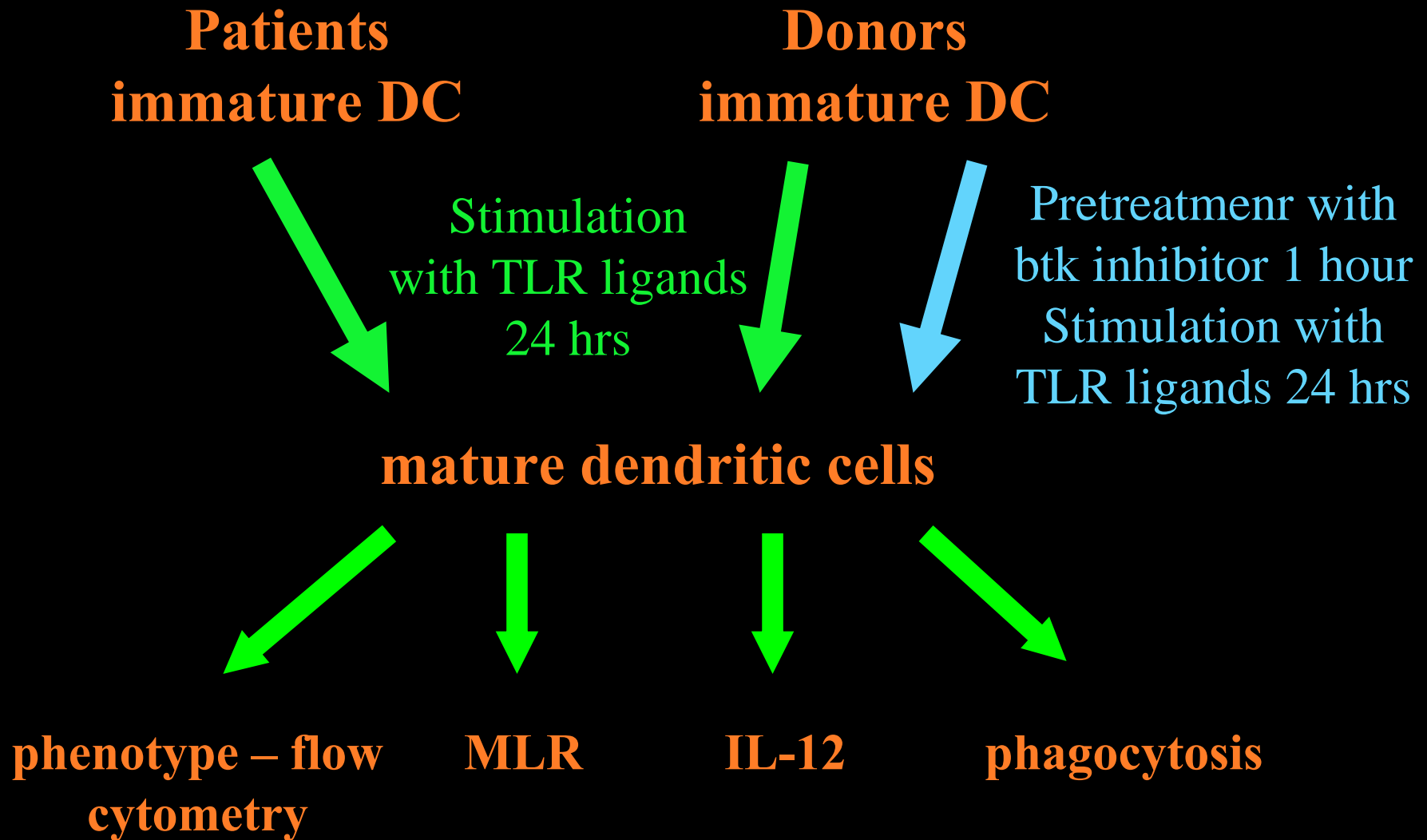
monocytes



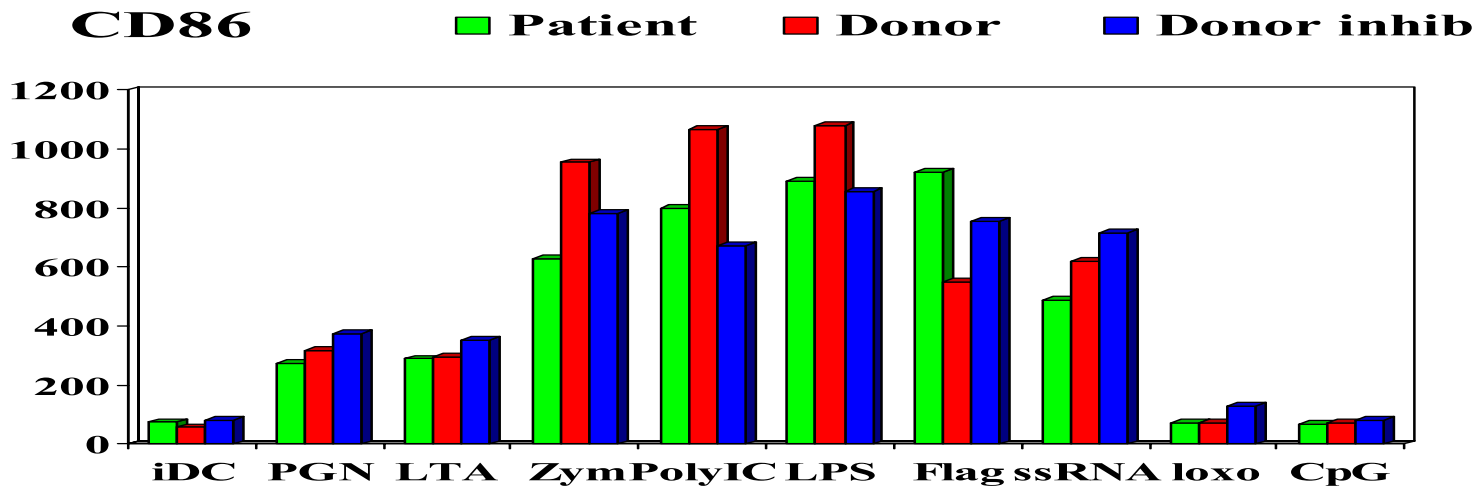
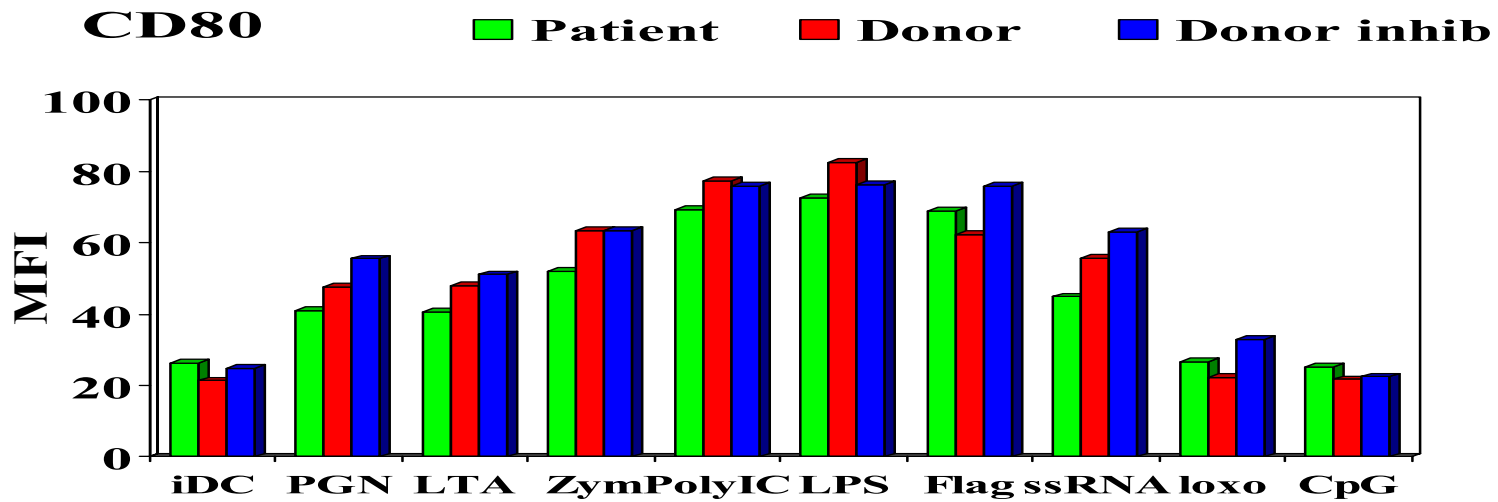
IL-4, GM-CSF
5 days, 37°C, 5% CO₂

immature dendritic cells

DC stimulation and functional tests



Phenotype CD80 and CD86



TLR2/6

TLR3

TLR4

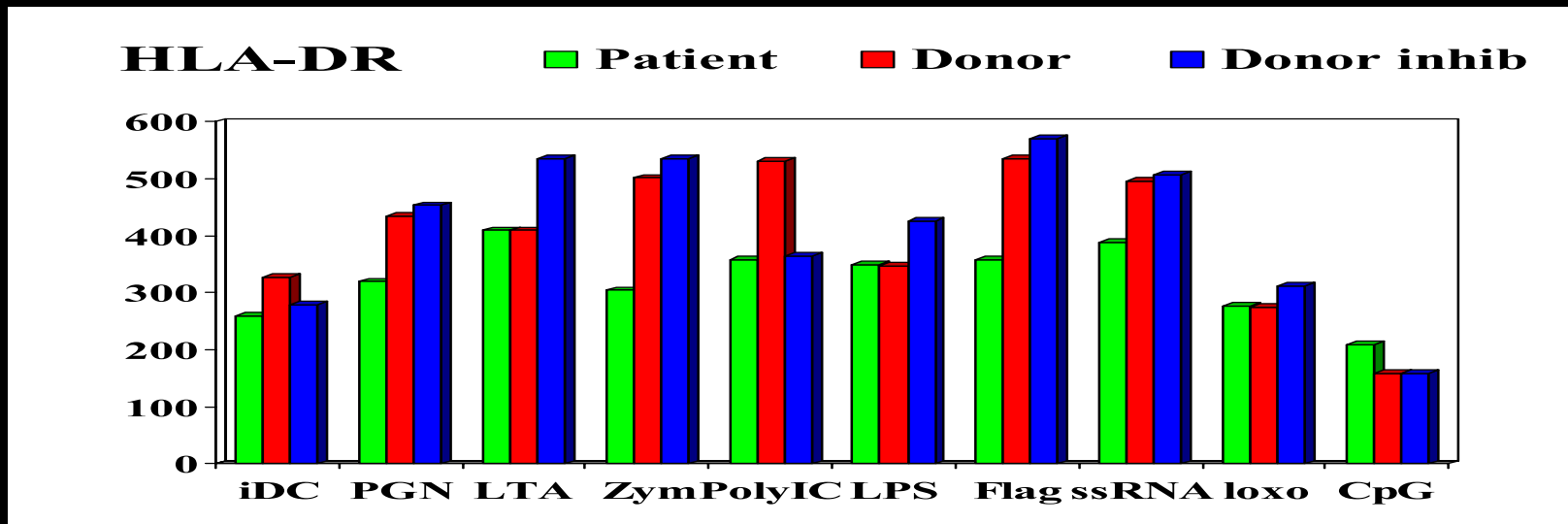
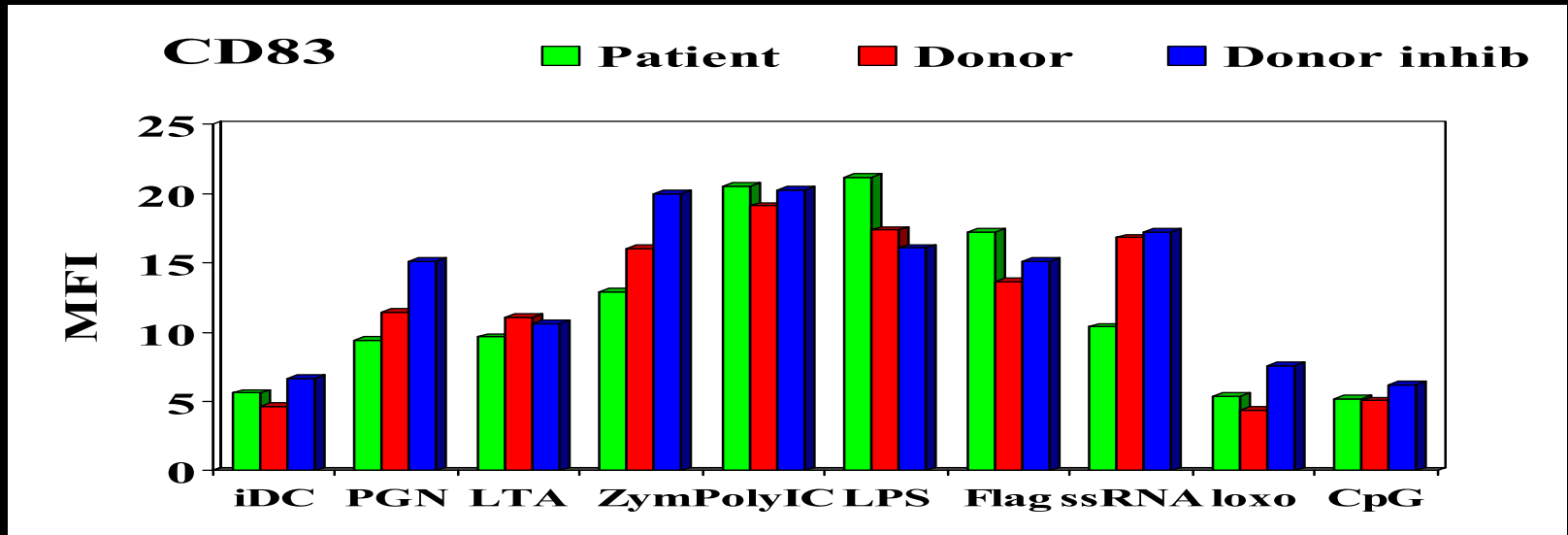
TLR5

TLR7/8

TLR7

TLR9

Phenotype CD83 and HLA-DR



TLR2/6

TLR3

TLR4

TLR5

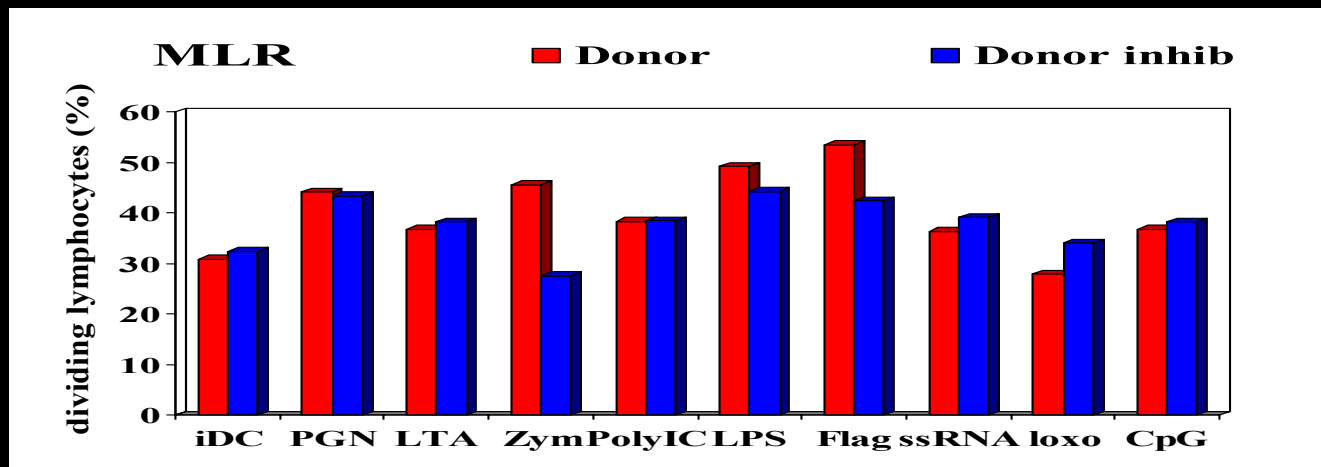
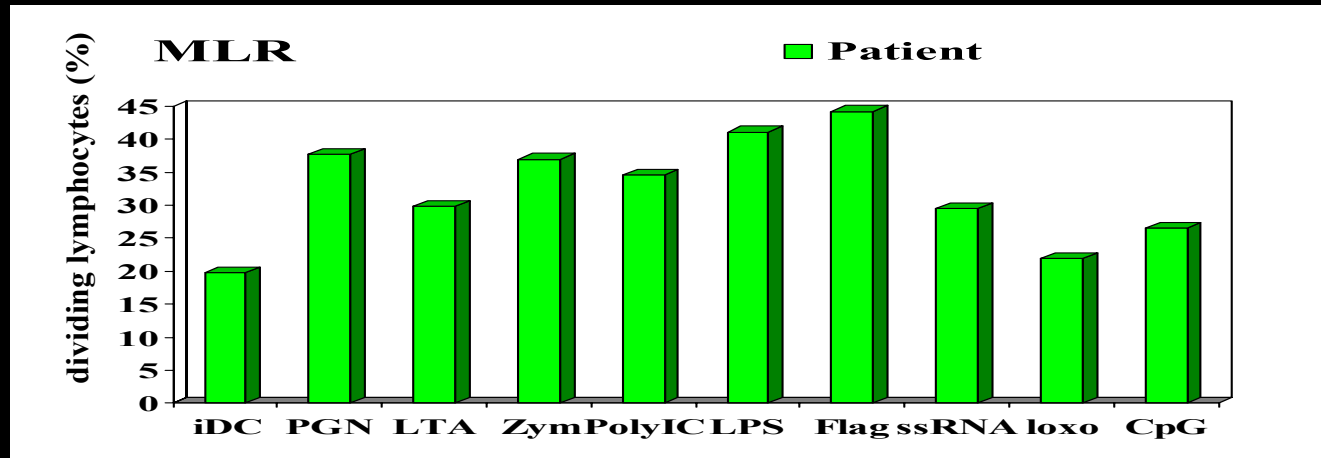
TLR7/8

TLR7

TLR9

Potential to stimulate T cells

Mixed Lymphocyte Reaction



TLR2/6

3

4

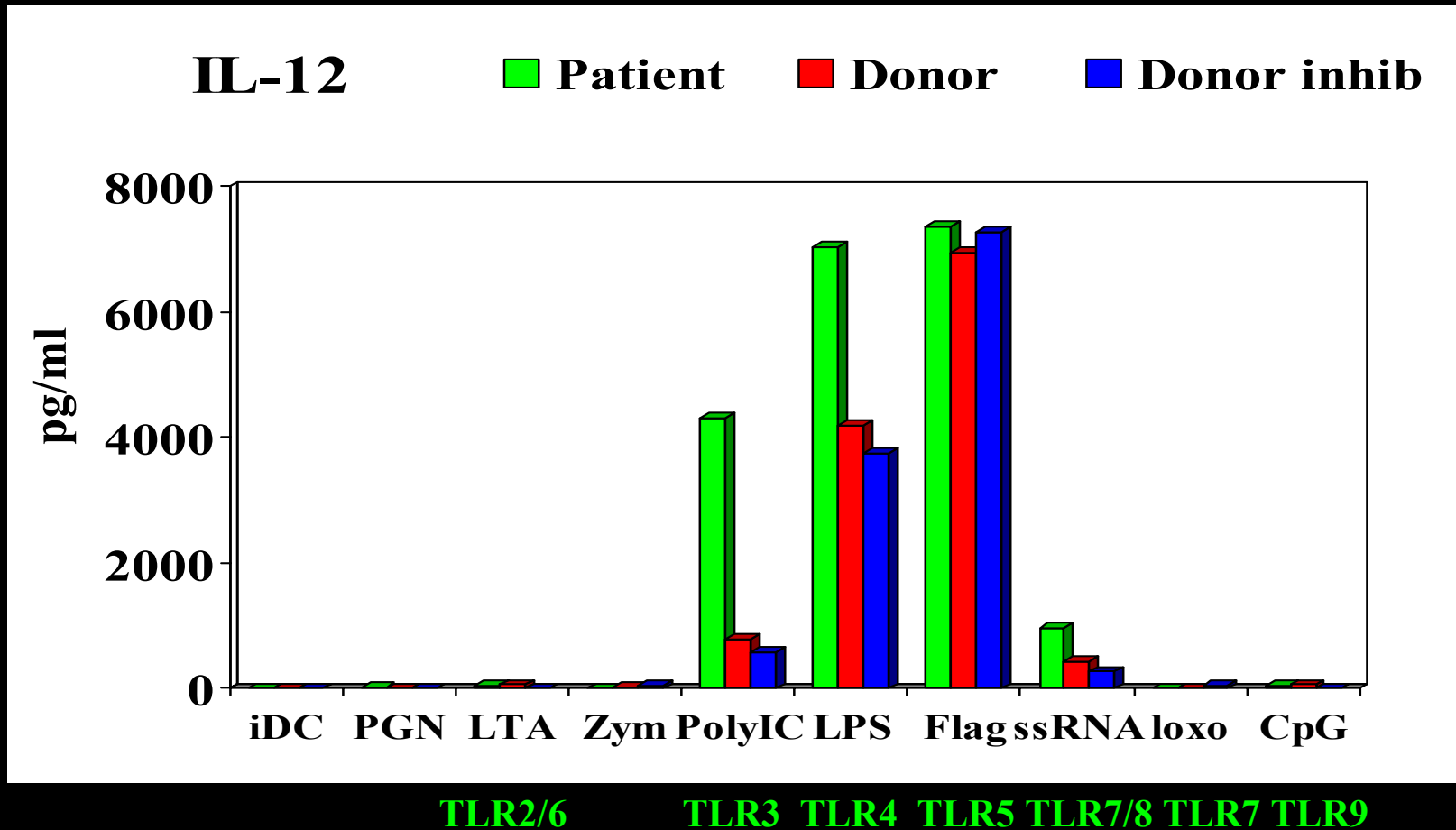
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7/8

7

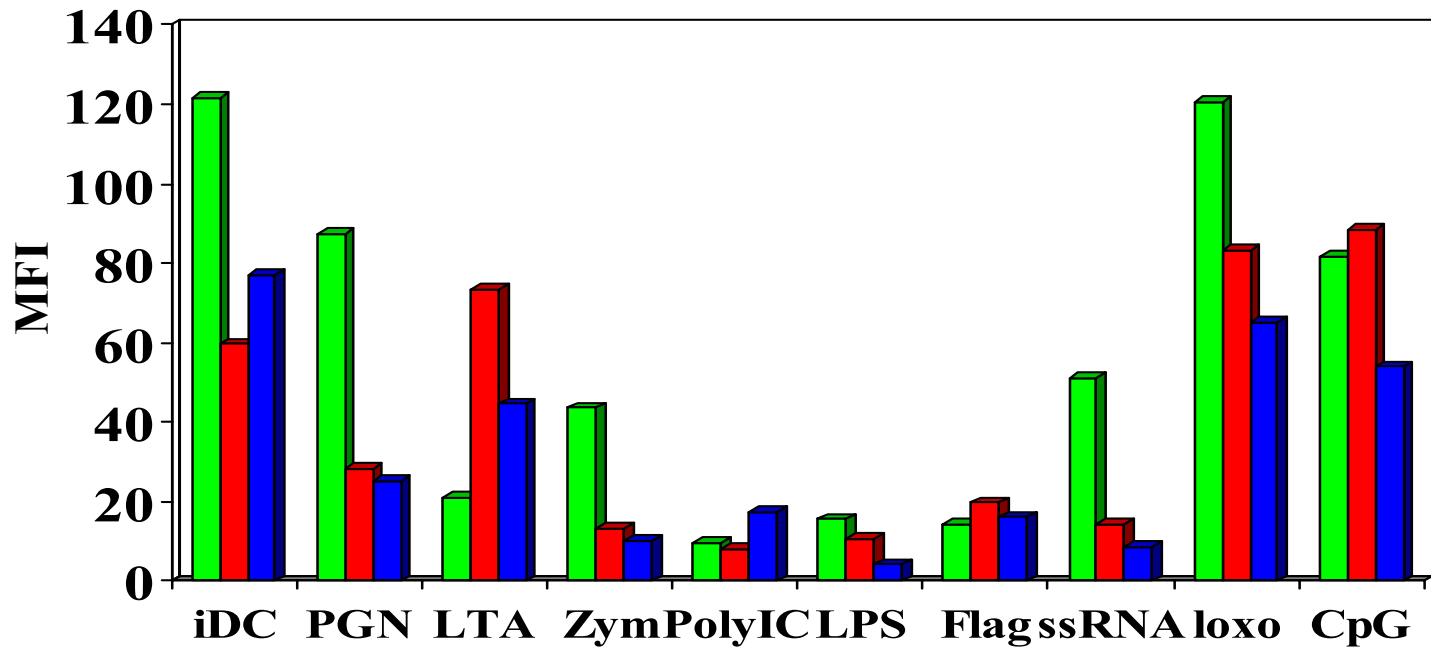
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IL-12 production ELISA



Phagocytosis of FITC-dextran

Phagocytosis ■ Patient ■ Donor ■ Donor inhib



TLR2/6

TRL3

TLR4

TLR5

TLR7/8

TLR7

TLR9

Conclusion

- **Mutation in btk does not influence:**
 - **phenotype**
 - **potential to stimulate T cell**
 - **IL-12 production**
 - **phagocytic activity**

in myeloid dendritic cells after TLR stimulation

Conclusion

- **Loxoribine (TLR7) and CpG (TLR9) do not trigger the maturation of myeloid DC**
- **Only the stimulation with polyIC, LPS, flagellin and ssRNA lead to the production of IL-12**

Acknowledgement

