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Cross-regulation of host STATs for the survival of *Toxoplasma gondii*

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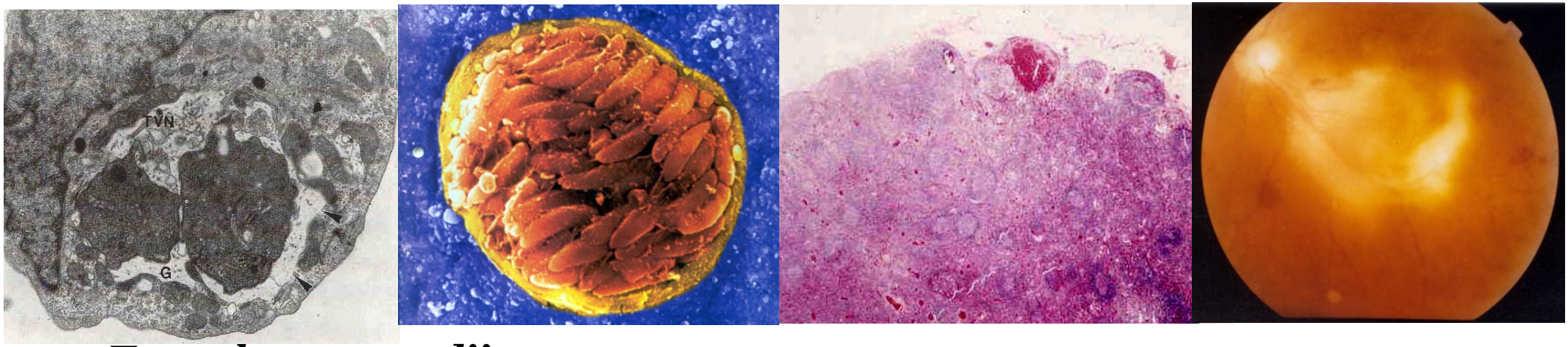
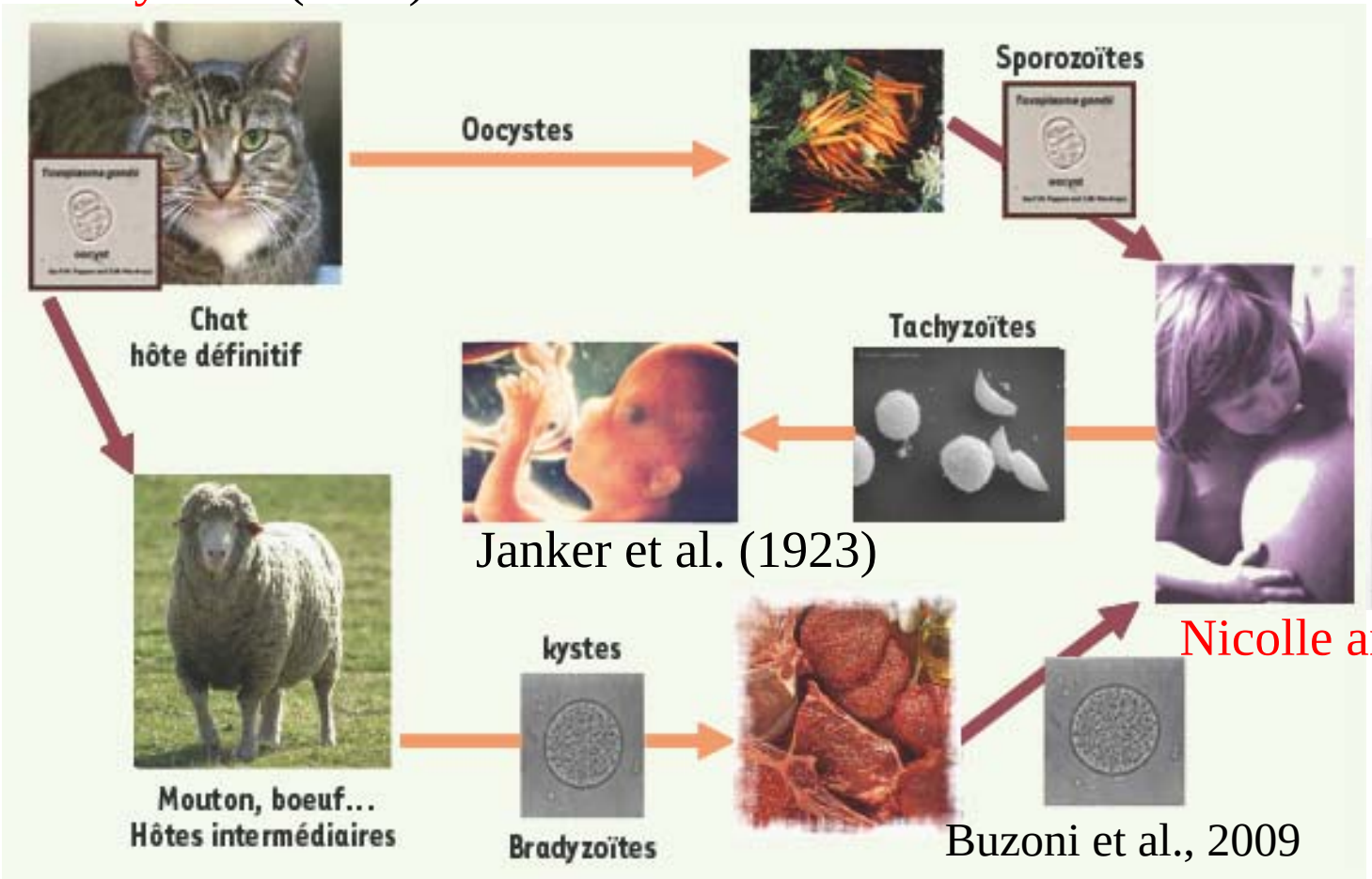


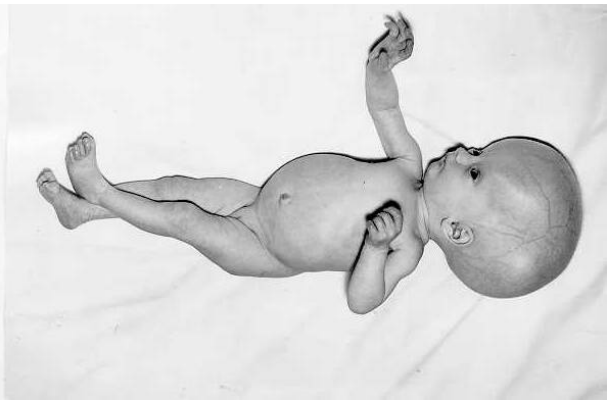
Toxoplasma gondii

: Intracellular protozoan that infects CNS through the invaion into macrophages, zoonotic and opportunistic

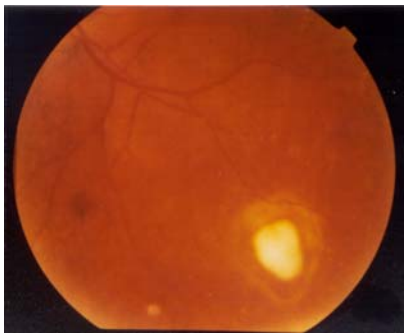
Life cycle of *Toxoplasma* and infection routes

Dubey et al. (1970)





Nicolle and Manceaux (1909)

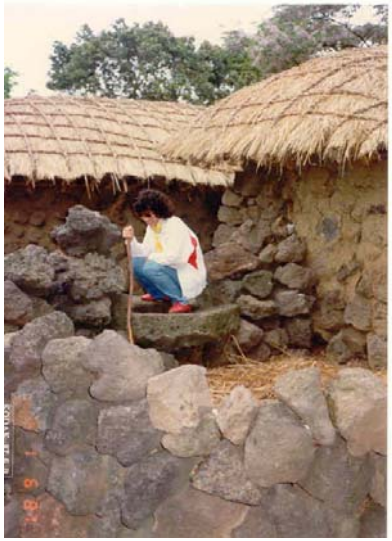


- **Zoonotic infection into herbivore and carnivores all over the world**
- **Infections almost benign or no symptom (?), but opportunistic**

Seroprevalence against *Toxoplasma* in Korea

Soh et al	1960	5.6% in 373 Korean	Skin test
Kim & Choi	1983	7.2% in 874 patients	ILA
Choi et al	1989	1.9% in patients	ILA
		5.8% in Cheju pts	
Choi et al	1992	7.0% in sera	ELISA
		5.6% in paired CSF	
Kim et al*	1993	Laboratory infection case	KJI:25
Kook et al	1997	7.7% in 542 children	ELISA
Choi et al	1997	Chorioretinites & lymphadenites	JID:175
Yang et al	2000	5.5% in 4,570 schools	ELISA
		12.9% in 474 adults in Cheju island	
Kim et al	2000	Recurrent uveitis	KJP:38
Song et al.	2005	0.79% in 5,175 sera of pregnant women	ELISA
		1.33% in 750 amniotic fluids	
Nam et al	2010	13.2% in 2,348 Jeju patients	ELISA

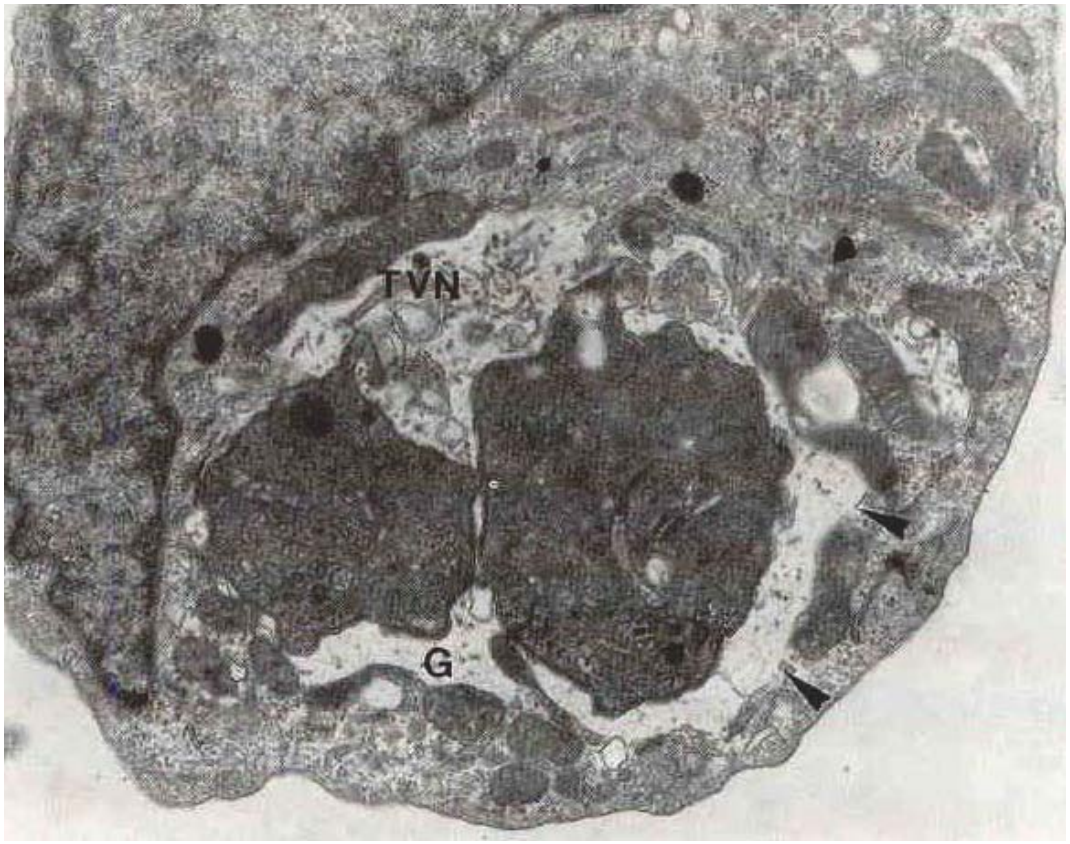
*: cases diagnosed and treated as toxoplasmoses

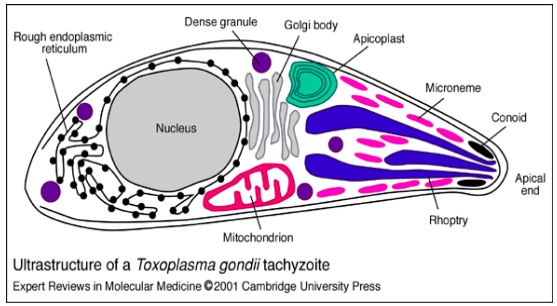


Human-pork-cat

Felids-water-human

Relationship between *Toxoplasma* and host cell





Ultrastructure of a *Toxoplasma gondii* tachyzoite
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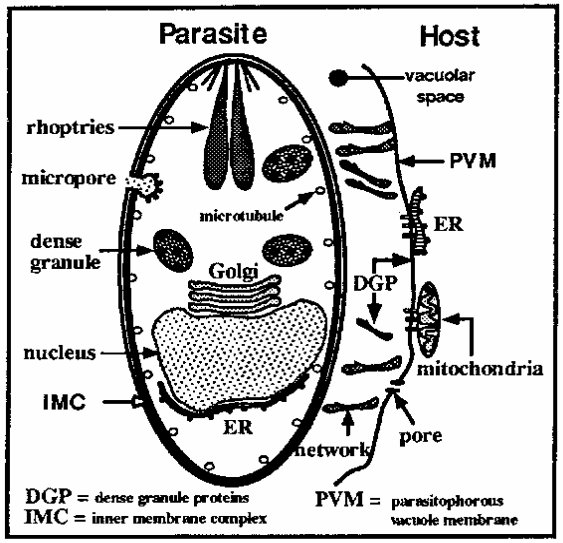
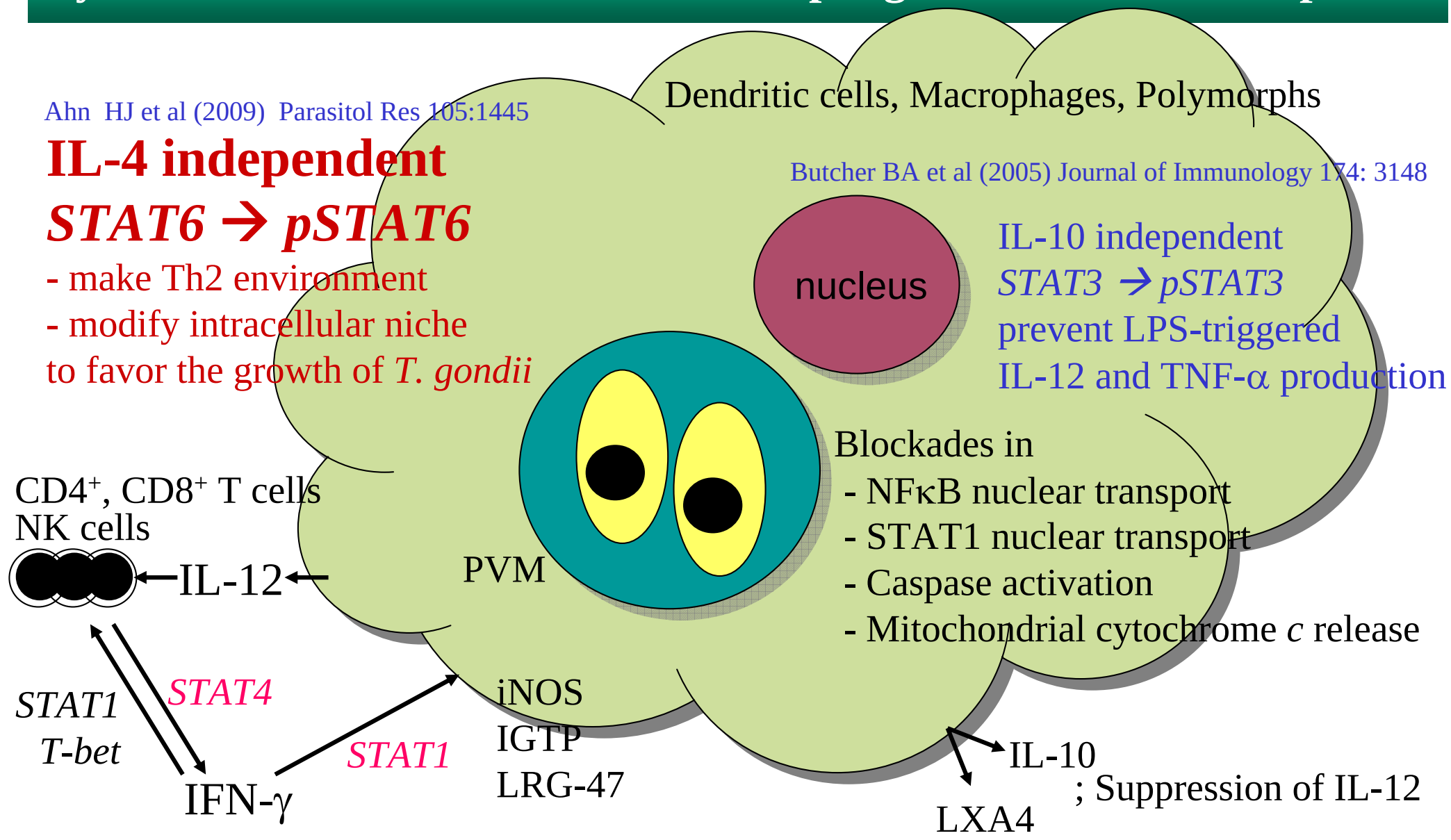


- *Toxoplasma* makes a border membrane (parasitophorous vacuolar membrane, PVM) in the margin of host cytoplasm,
- secretes proteins from organelles (MIC, ROP, and DG) to decorate PVM .
- Under the TEM, host mitochondria and ER are recruited to the PVM.

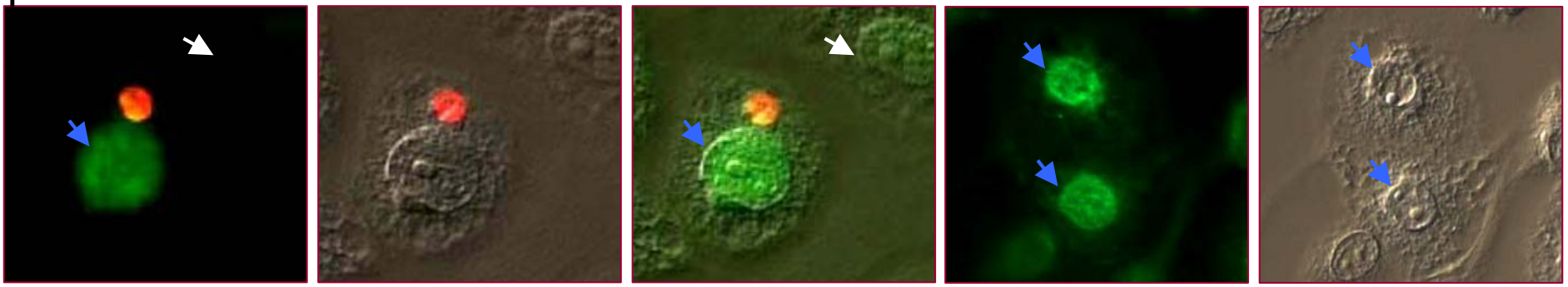
Cytokines/STATs in/around the macrophage infected with *Toxoplasma*



Denkers EY (2003) FEMS Immunology and Medical Microbiology 39: 193

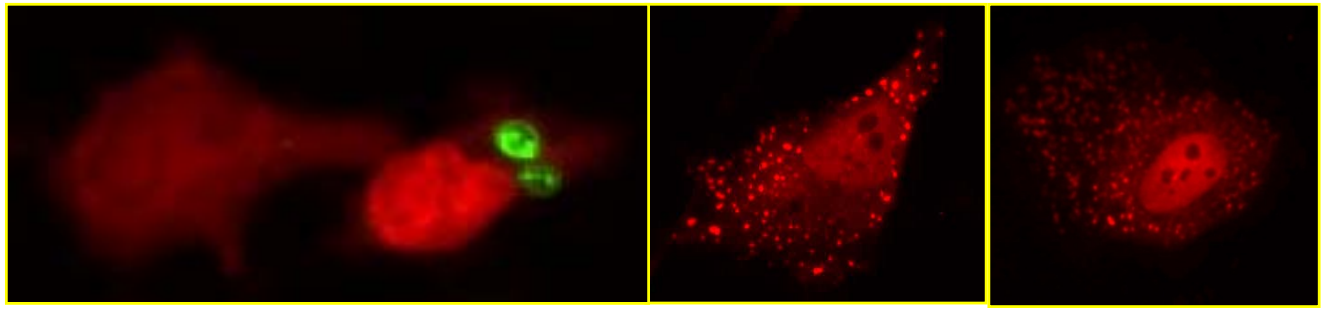
➢IFA with rabbit pSTAT6 pAb (FITC) and mouse GRA3 mAb (TRITC)

pSTAT6/GRA3 GRA3 Phase

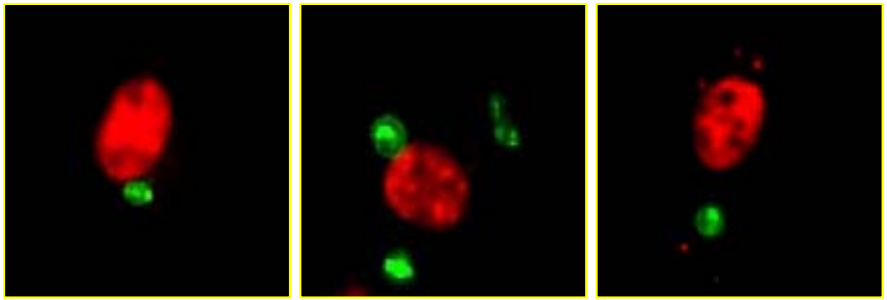


THP-1 + *T. gondii* THP-1 + IL-4

➢Transfection assay with exogenous pDs2Red-STAT6

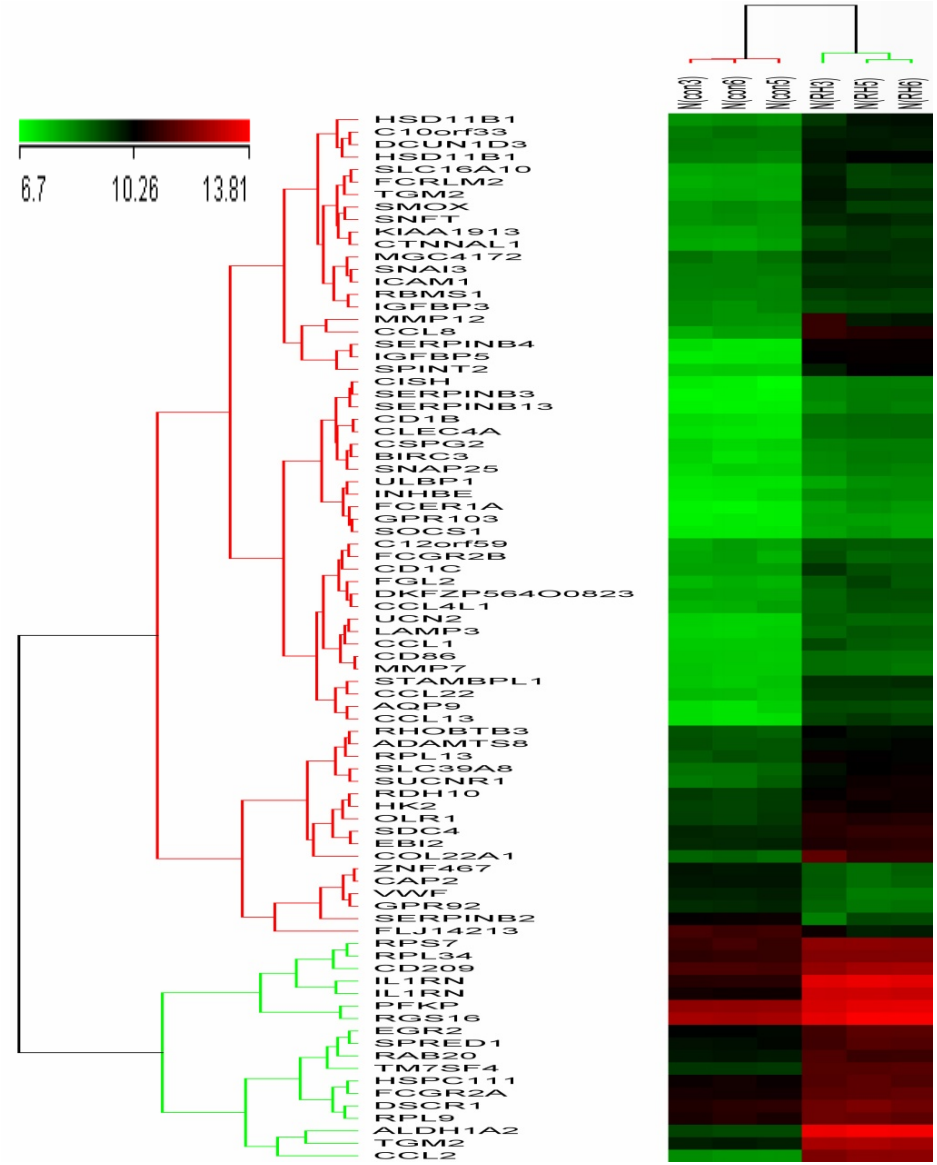


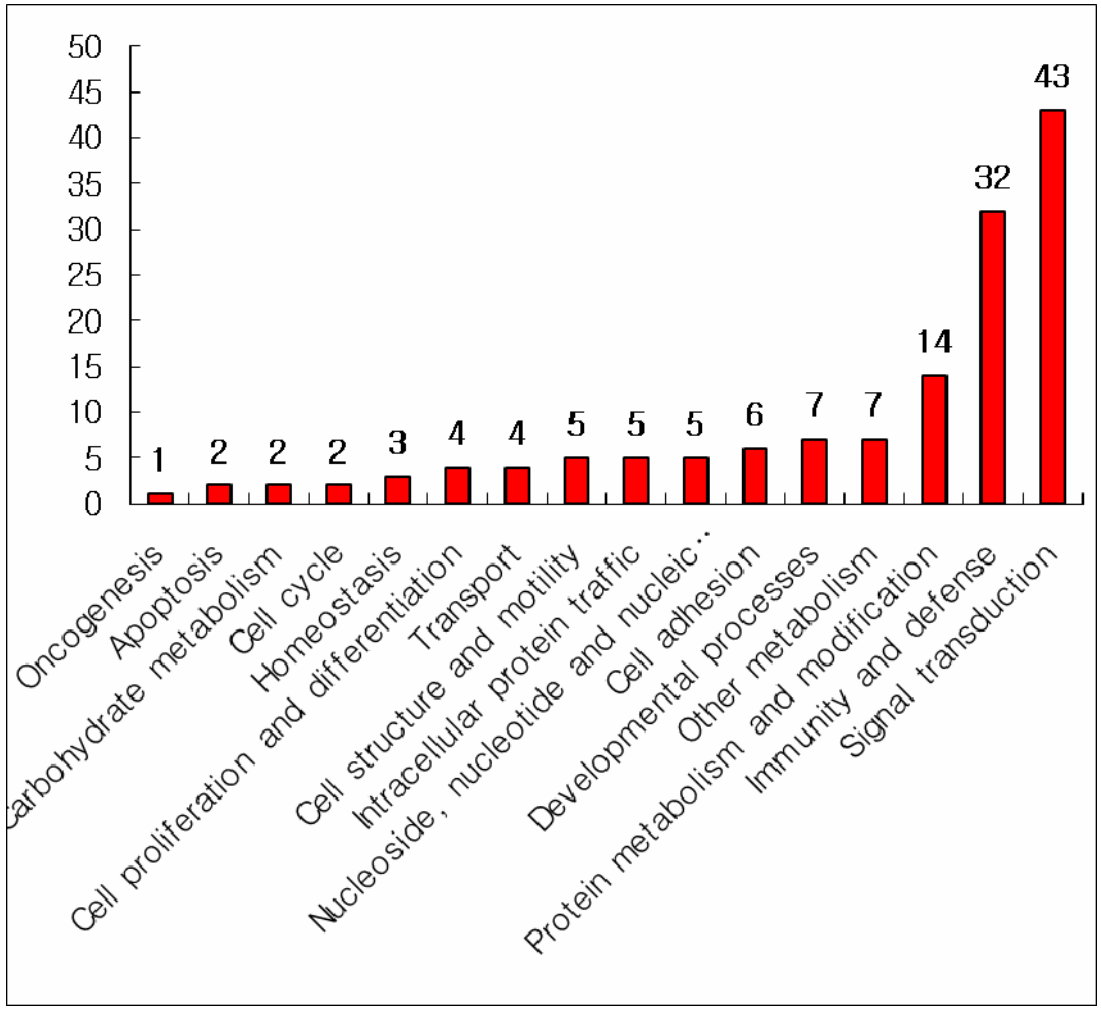
HeLa + *T. gondii* (mAb GRA3) HeLa + IL-4



A549 HEK293 Vero76

Microarray analysis of *T. gondii* infected THP-1 cells





Among 12,850 probes used, 84 gene expressions change significantly after *T. gondii* infection.

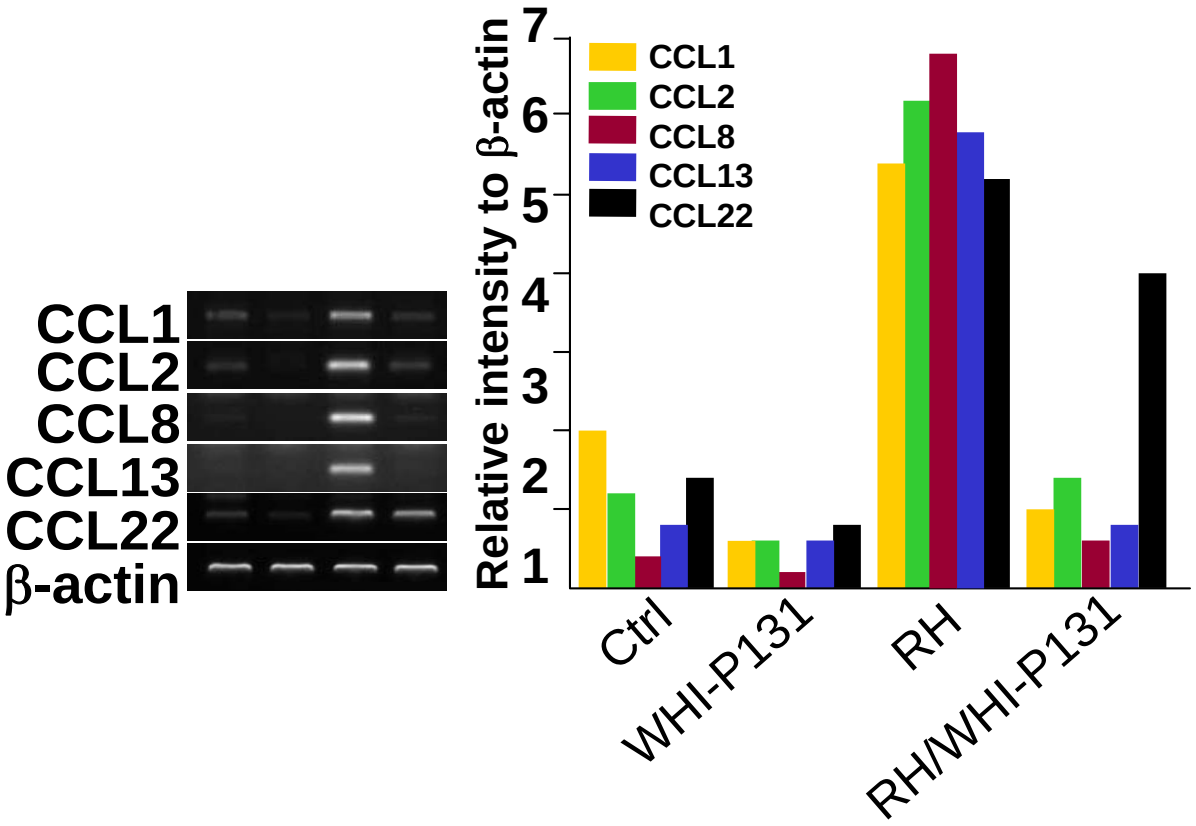
Cluster of cytokine/chemokine mediated immunity

Target	fold (infected/control)
CCL1 (I-139)	3.13
CCL2 (MCP-1)	15.97
CCL3	1.91
CCL3L1	1.28
CCL3L3	1.72
CCL4L1	2.31
CCL5	1.09
CCL7	1.52
CCL8 (MCP-2)	7.46
CCL13 (MCP-4)	4.92
CCL16	-1.02
CCL17	1.61
CCL20	1.76
CCL21	1.01
CCL22 (MDC)	3.79
CCL23	-1.10
CCL26	1.42
CCL28	-1.18

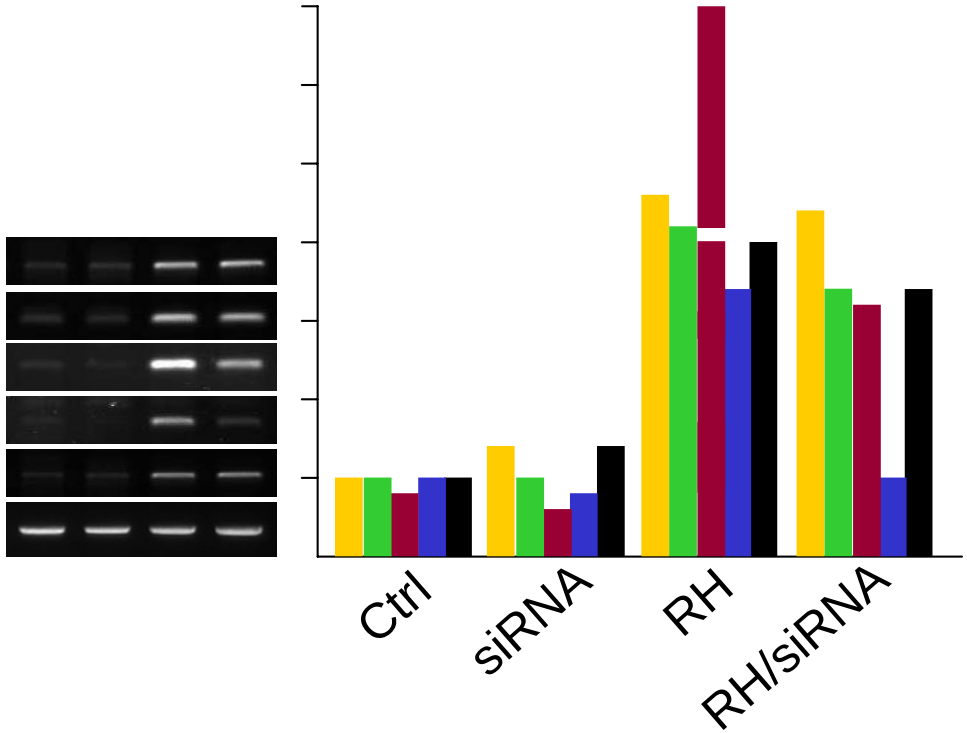
Among CCLs (chemokine (C-C motif) ligands),
6 CCLs were induced significantly,
but, in others and CXCLs (chemokine (C-X-C motif) ligands) no significant changes in expression.

Target	fold (infected/control)
CXCL1	-1.03
CXCL3	1.05
CXCL10	1.16
CXCL14	1.06
CXCL16	-1.27

Effect of JAK3 inhibitor (WHI-P131)



Effect of siRNA of STAT6



→ STAT6 mediated gene expressions

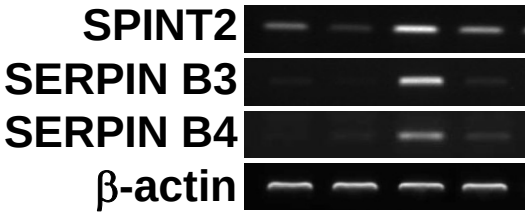
;Secreting those CCLs, *T. gondii* infected macrophage attracts more monocytes and Th2 cells of CCR3 and CCR4 to make, so called, the 'Th2 environment' nearby the infected macrophage.

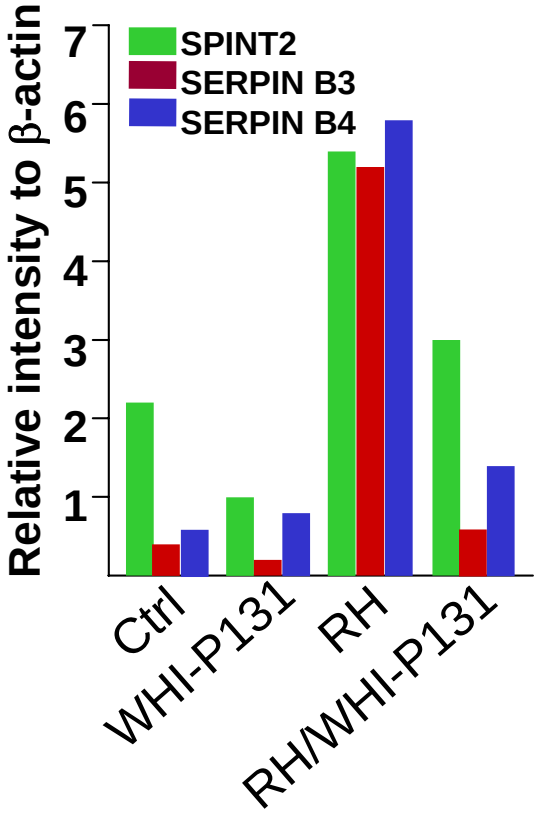
Cluster of proteolysis

Target	fold (infected/control)
SPINT2	6.65
SERPIN A1	-1.04
SERPIN A10	-1.04
SERPIN A12	1.07
SERPIN B1	1.03
SERPIN B2	-2.59
SERPIN B3	3.18
SERPIN B4	10.10
SERPIN B6	1.04
SERPIN B8	-1.16
SERPIN B13	2.86
SERPIN E1	-1.02
SERPIN E2	1.45
SERPIN F1	-1.42
SERPIN G1	1.00
SERPIN H1	1.02
SERPIN I1	-1.55

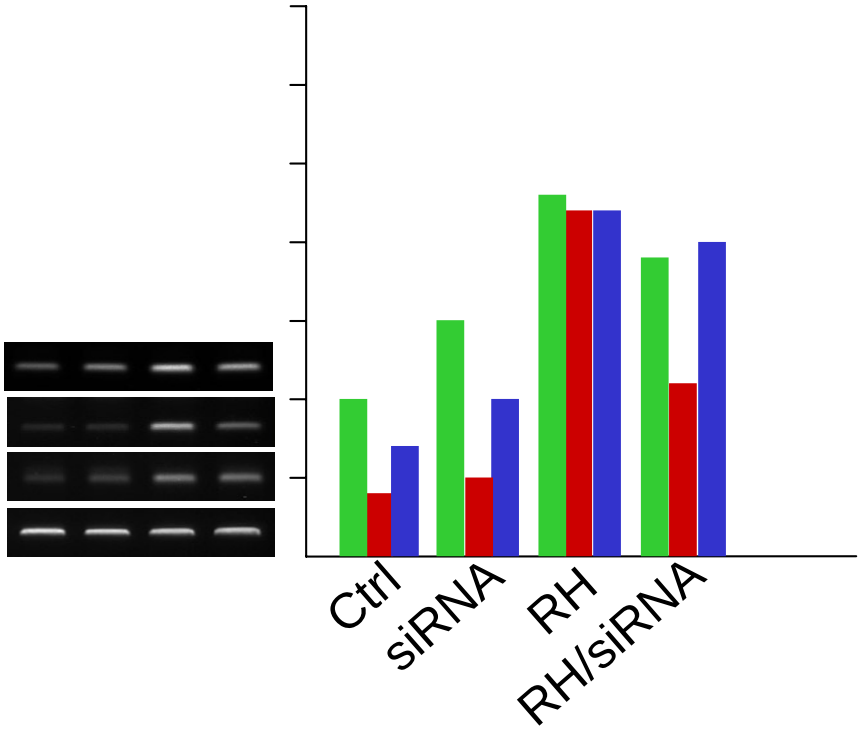
SERPIN B3, squamous cell carcinoma antigen-1 (SCCA-1); cross-class inhibition of Cathepsins L and V,
SERPIN B4, SCCA-2; cross-class inhibition of Cathepsin G and chymase,
SERPIN B13, Headpin (PI13); inhibition of Cathepsins L and K.
SERPINB2, Plasminogen activator inhibitor-2 (PAI2); inhibition of PA

Effect of JAK3 inhibitor (WHI-P131)





Effect of siRNA of STAT6



→ STAT6 mediated gene expressions

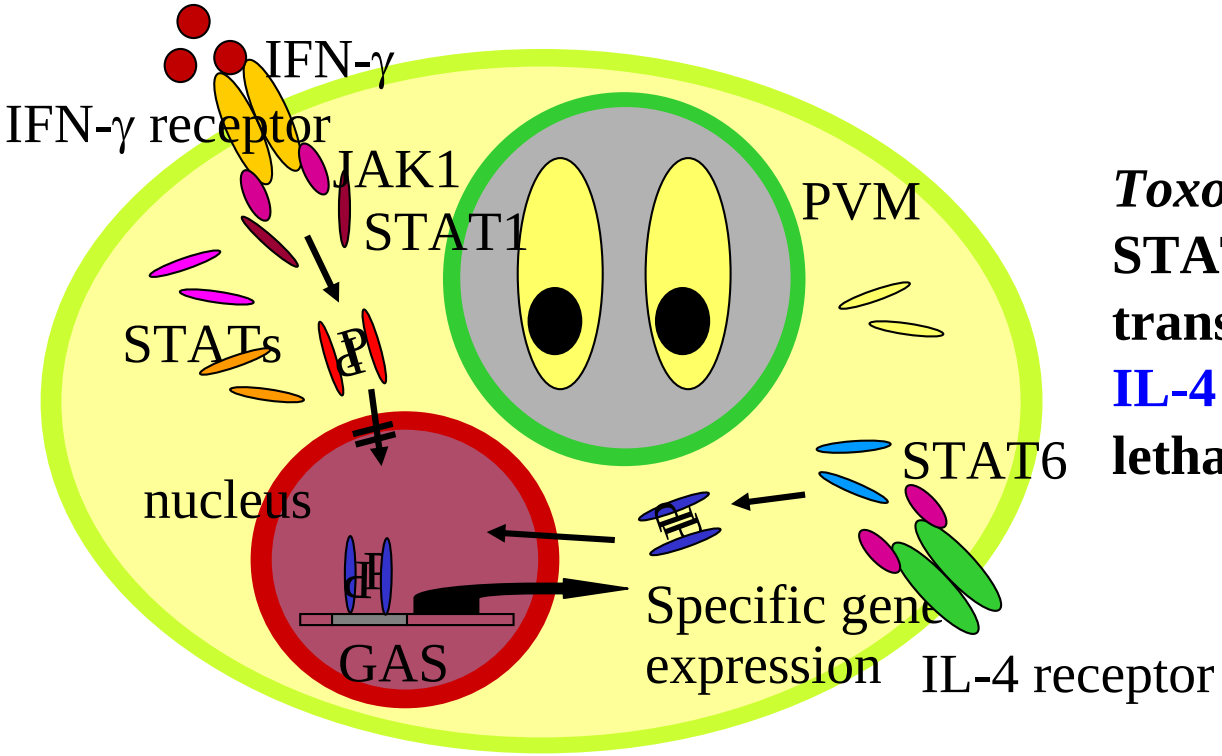
;The functions were not defined clearly, SPINT2, SERPIN B3, and B4 may participate in

i) the protection of intracellular damage from activated lysosomal enzymes,

ii) the inhibition of caspase activation to block the apoptosis,

iii) the inhibition of proteases of antigen presentation to reduce the antigenic recognition, etc.

STAT cross-regulation within *Toxoplasma*-infected macrophage

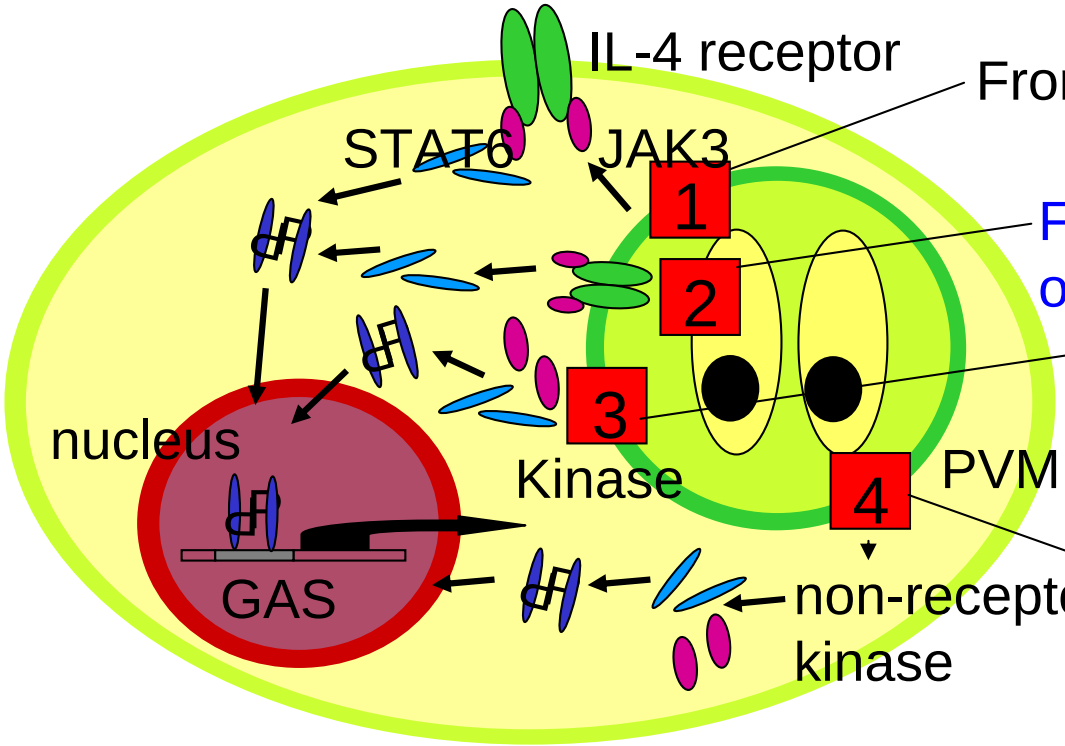


***Toxoplasma* infection makes the host STAT6 phosphorylated and translocated into the nucleus without IL-4 stimulus, and then copes with the lethal actions by IFN-γ.**

→**"if the cells are infected first, subsequent exposure to IFN-γ is unable to control the infection and the parasite grows normally."**

→**Cross-regulation of STAT level occurs just in the infected cells but not in uninfected cells, which suggests that the survival strategy of the intracellular *T. gondii* may prevent the infected individual from unexpected action of cross cytokine, IL-4.**

Where's the kinasing activity to STAT6 come from?

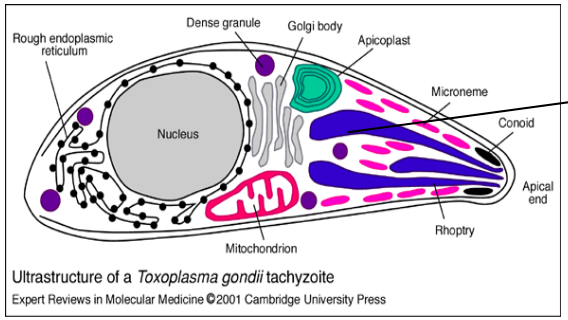


From JAK3 attached to IL-4 receptor

From IL-4 receptor-like molecule on the PVM

From kinase(s) of *Toxoplasma* secreted to the PVM

From non-receptor kinase of cytosol



Ultrastructure of a *Toxoplasma gondii* tachyzoite

Among the rhoptry protein (ROP1-21), ROP2, 4, 5, 7, 8, 11, 16, 17 and18 have kinase domains in their C-terminal halves.

Prospects

- ✓ **Understand the STAT level cross –regulation and the underlying mechanism of STAT signal transduction, why and how?**
- ✓ **Find therapeutic targets with optimized IUD or small molecules.**
- ✓ **Provide research model of infection and defense for those intracellular protozoans such as Theileria, Neospora, or others.**