Cell death and immune responses

I - Genetic regulation of cell death

II - Deregulation and pathologies

III - Bcl-2 targetting and cancer

IV- Cross-presentation of dead cells

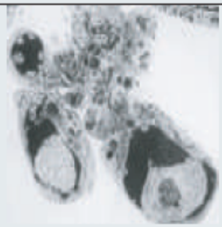
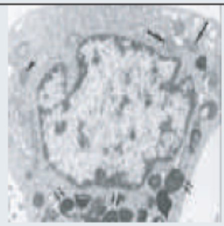
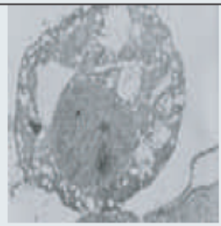
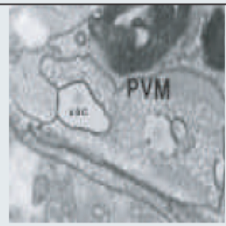
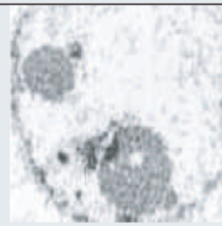
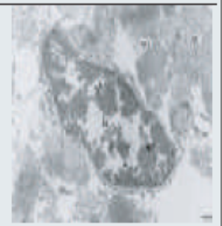
- **- Phagocytes**
- **- Tolerance versus activation**

Inserm

Y. LEVERRIER, Inserm U851

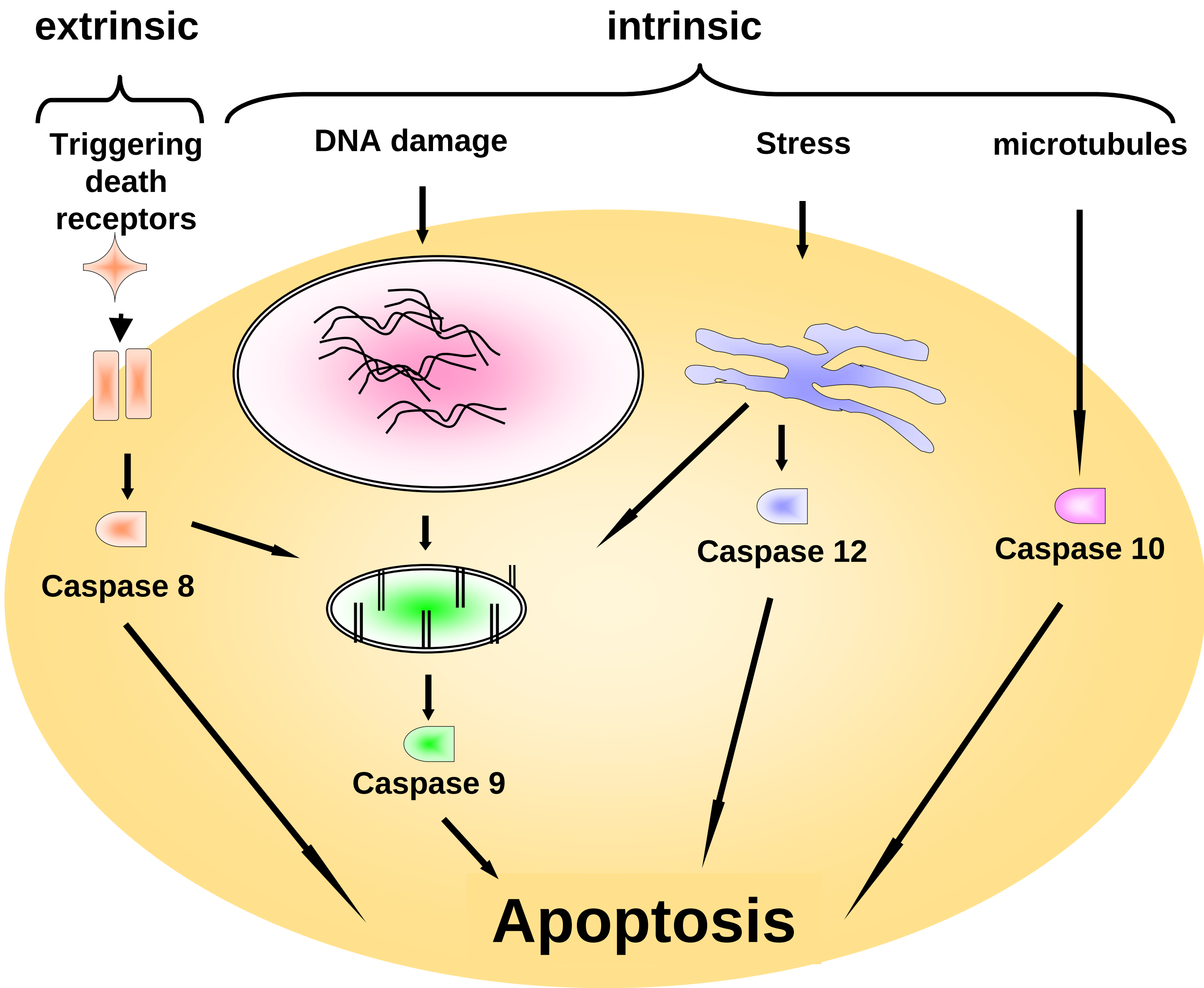
Multiple forms of cell death

Table 1 | Characteristics of dying cells

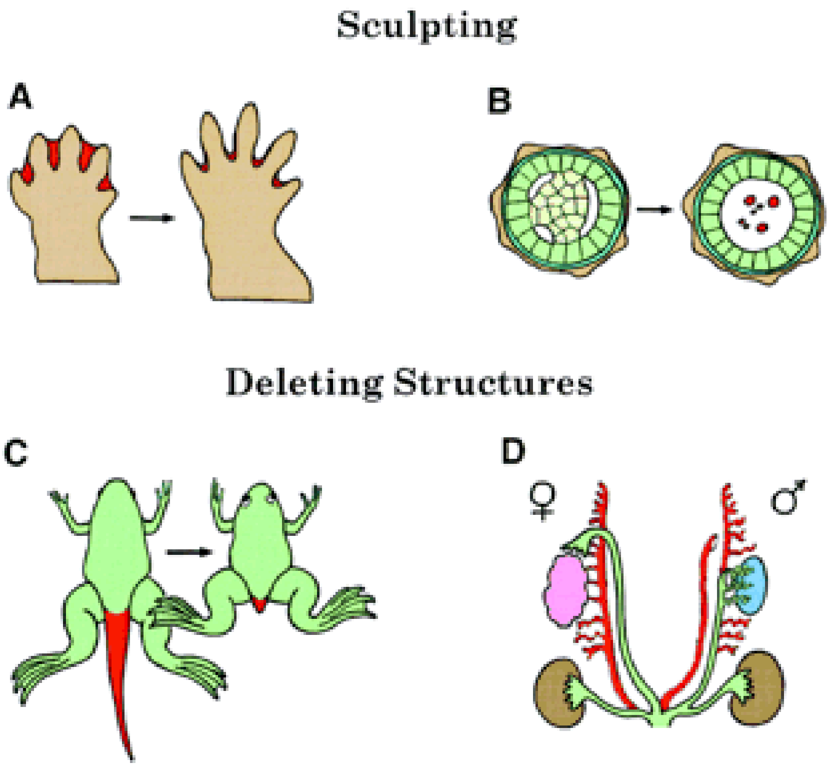
Characteristics	Established forms of cell death		Atypical forms of cell death			
	Apoptosis	Autophagic	Paraptosis	Calcium-mediated	AIF/PARP-dependent	Oncosis
Morphology						
	Chromatin condensation, nuclear fragmentation, apoptotic bodies	Autophagic vacuoles	ER swelling, mitochondrial swelling	Membrane whorls	Mild chromatin condensation	Cellular swelling
Triggers	Include death receptors, trophic-factor withdrawal, DNA damage, viral infections	Serum, amino-acid starvation, protein aggregates	Trophotoxicity	Calcium entry, *C. elegans deg* mutants	DNA damage, glutamate, nitric oxide	Ischaemia, excitotoxicity
Mediators	Caspases, BH1-3, BH3 proteins	JNK1? MKK7? ATG orthologues	ERK2, NUR77	Calpains, cathepsins	PARP, AIF	JNK
Inhibitors	Caspase inhibitors, BH1-4 proteins	JNK inhibitors?	U0126 (MEK), DN NUR77	Calreticulin, Some calpain inhibitors?	PARP inhibitors	JNK inhibitors
Examples	Type I PCD, nuclear PCD	Type II PCD	Type III PCD, cytoplasmic PCD	*C. elegans deg* mutants	Some excitotoxic PCD	Ischaemic PCD

Note the difference in morphology present in each form, as well as the differences in biochemical mediators, inducers, and inhibitors. At present, only apoptosis and autophagic PCD are generally accepted as being legitimate forms of PCD; however, ongoing research should reveal which of the additional candidates represent novel pathways of PCD. DN, dominant-negative; DEG, degeneration; U0126, a mitogen-activated protein kinase inhibitor. (Images reproduced, with permission, from refs 58-62.)

Multiple forms of apoptosis



Apoptosis *in vivo*



Jacobson, Weil and Raff, Cell 1997

Adult:

- **10^{10}** cells die every day

Self:

- Adjusting cell numbers
- T cell selection in the thymus
- Neutrophils cell death (end of inflammation)

Mutated self:

- Tumor cells

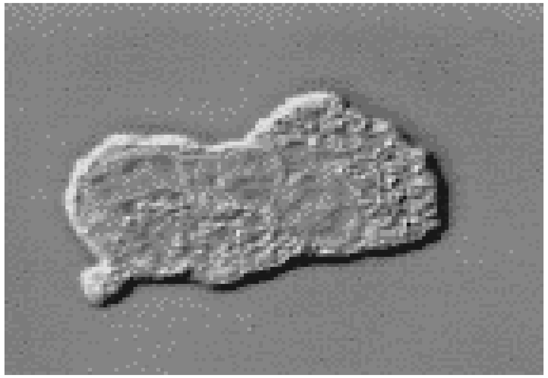
Self + Non Self:

- Infected cells (virus, bacteria)

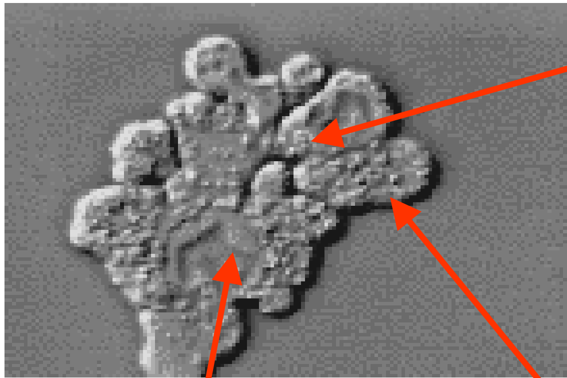
Apoptosis: morphology

‘Apoptosis’ (Kerr, Wyllie and Currie, 1972)

Live neutrophil



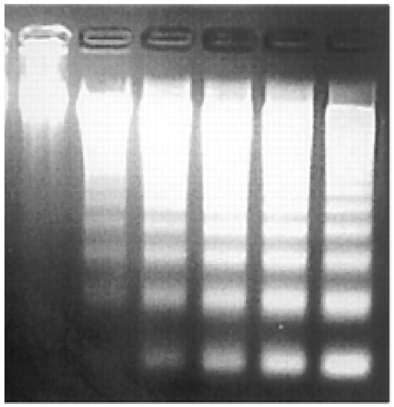
Apoptotic neutrophil

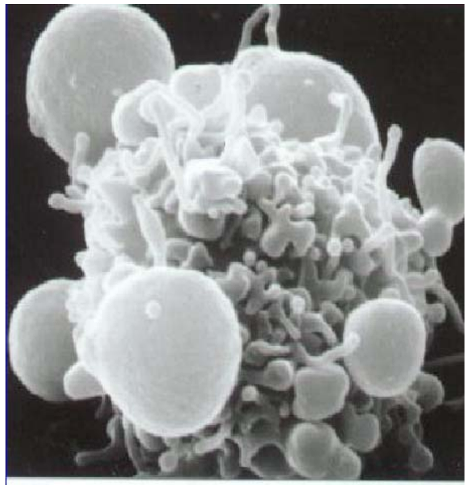


Cytoplasm condensation and fragmentation => apoptotic bodies

Chromatin condensation and nucleus fragmentation

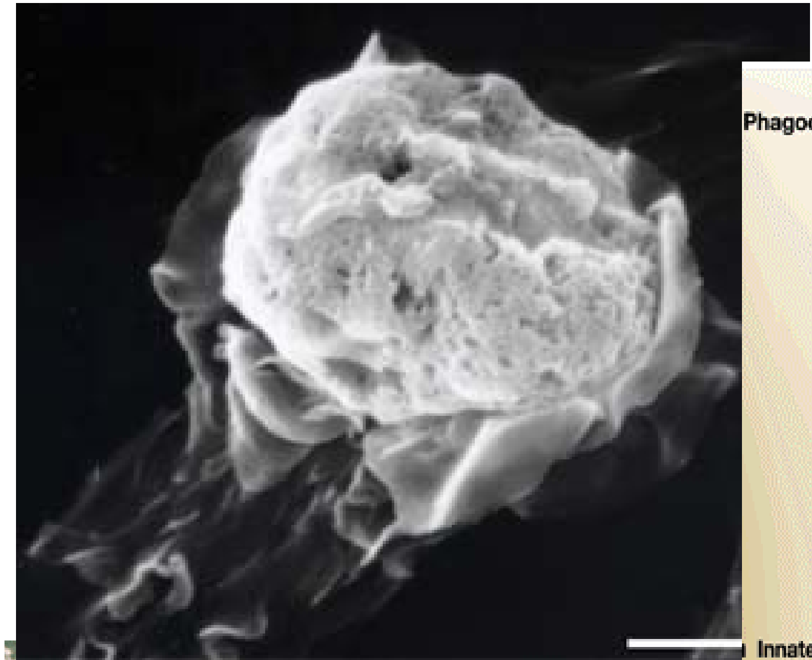
Blebbing

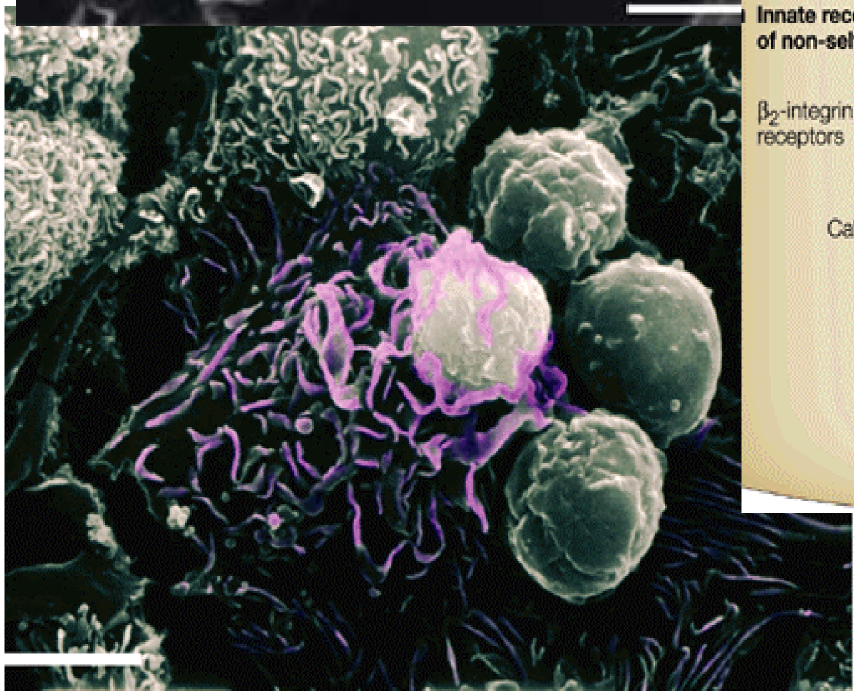


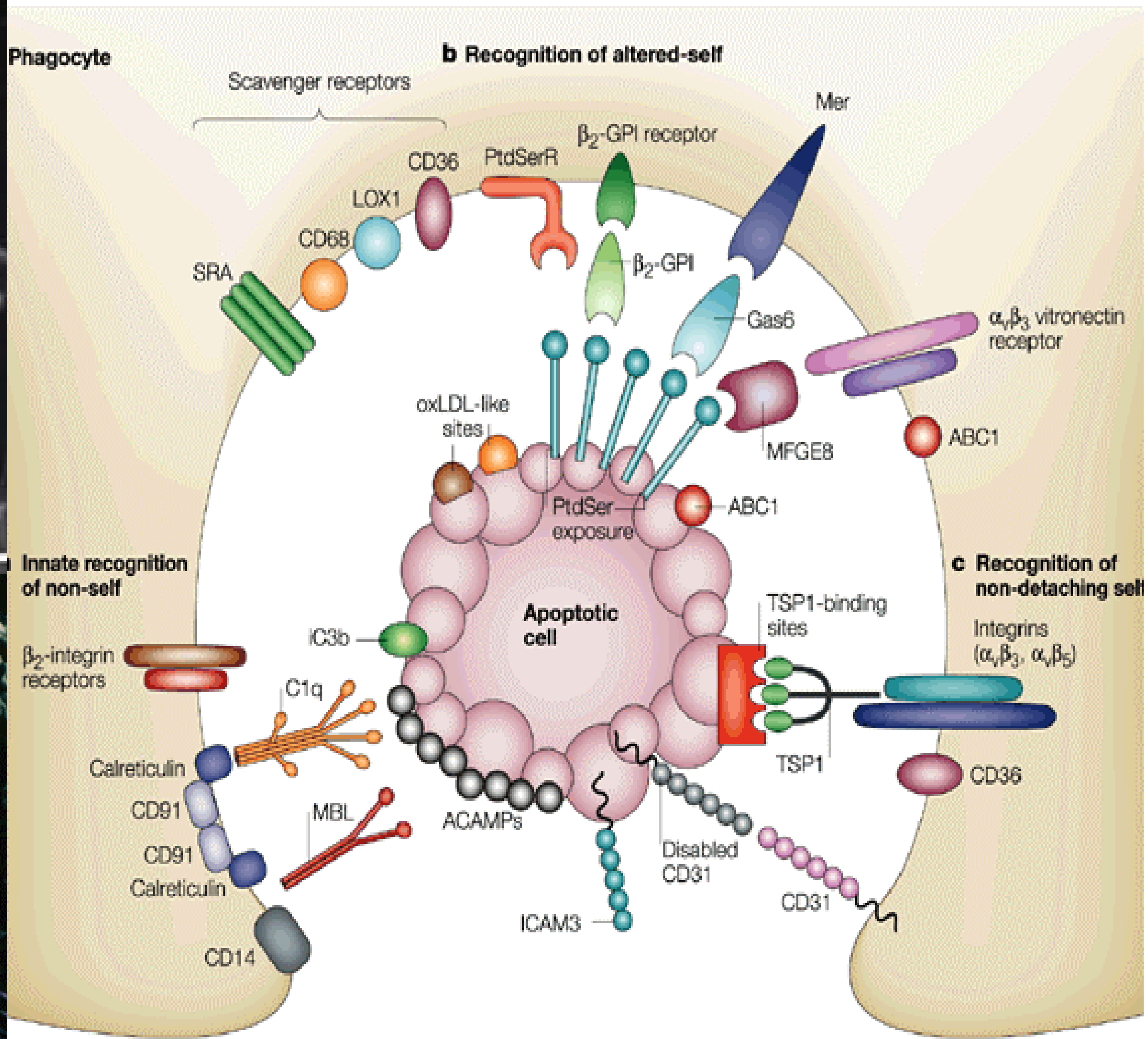


- ✔ Organelles are intact
- ✔ Plasma membrane is intact

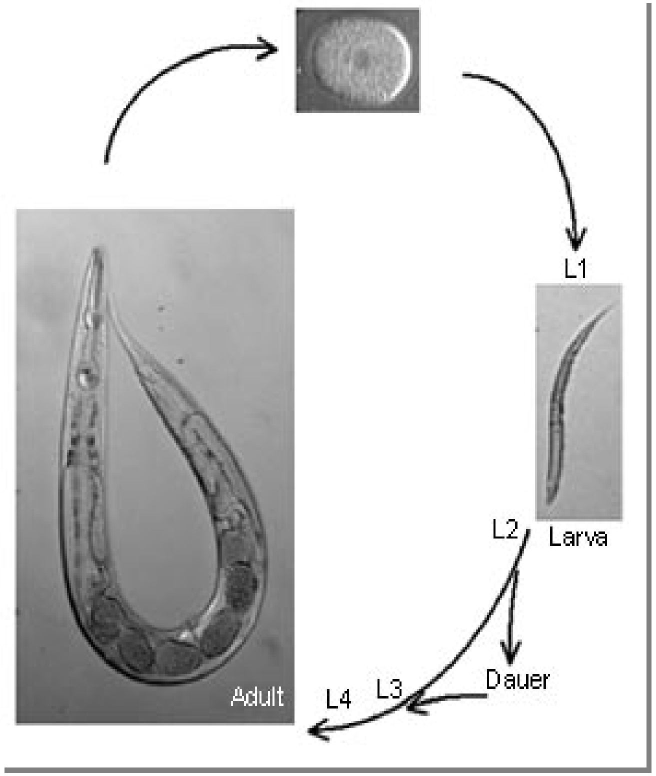
Dead cells express "eat me" signals

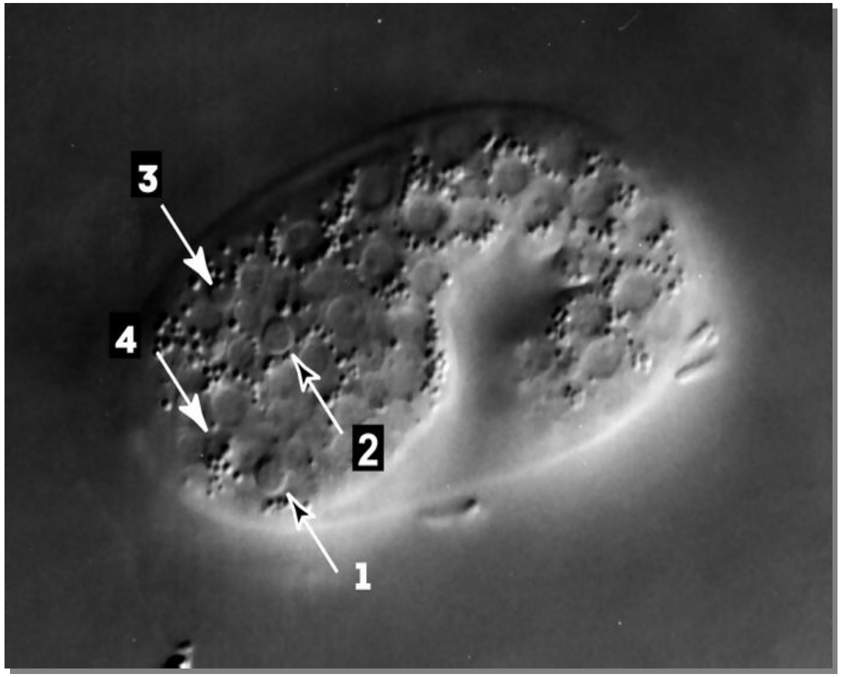






Genetic regulation of cell death: *C. elegans*





Nobel prize Medecine 2002

Sydney Brenner:	Nematode to identify genes that control cell biology
John Sulston:	Lineage (1976)
Robert Horvitz:	Mutagenesis to identify genes regulting cell death in *C.elegans*

Genetic regulation of cell death: *C. elegans*

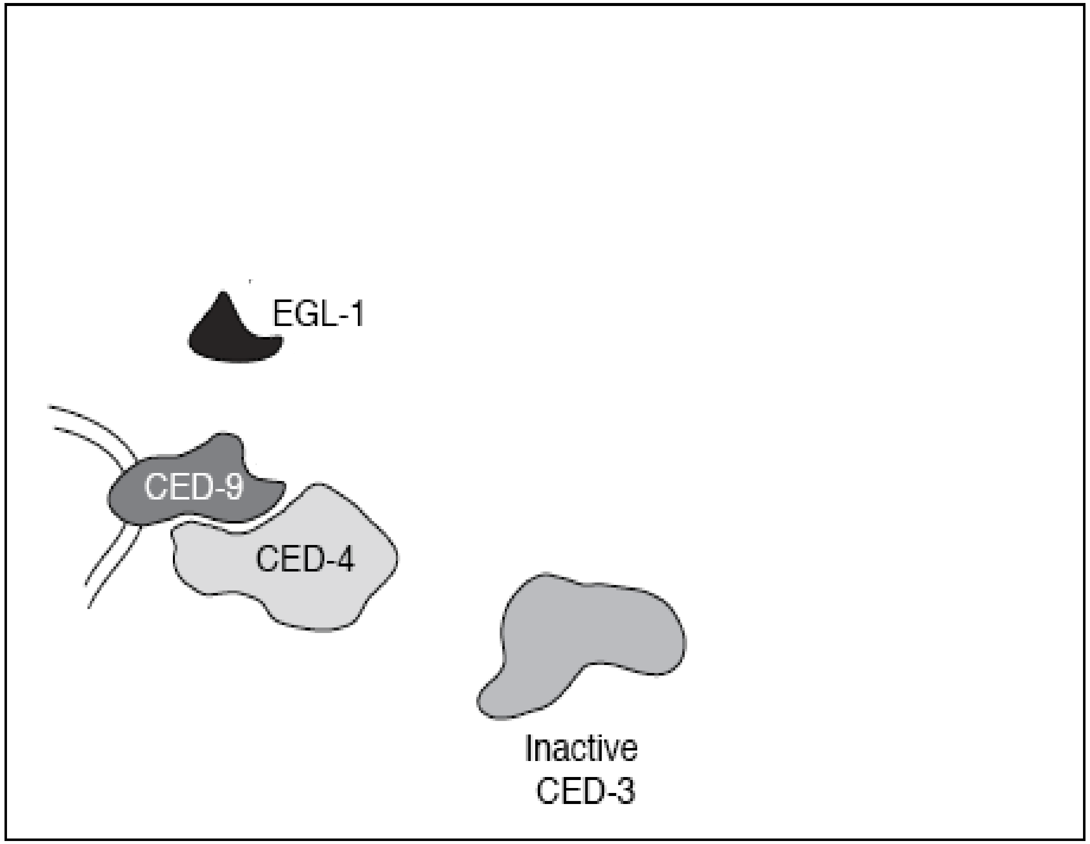
Genotype	Phenotype	
	Viable?	Programmed cell death
Wild-type	Yes	Normal
ced-9(lf)	No	Excess
ced-4(lf)	Yes	Reduced
ced-3(lf)	Yes	Reduced
egl-1(lf)	Yes	Reduced
ced-4(lf);*ced-9*(lf)	Yes	Reduced
ced-9(lf);*ced-3*(lf)	Yes	Reduced
ced-9(lf);*egl-1*(lf)	No	Excess

ced-9 ⊣ PCD
ced-4 → PCD
ced-3 → PCD
egl-1 → PCD
ced-9 - ced-4 → PCD
ced-9 - ced-3 → PCD
egl-1 - ced-9 ⊣ PCD

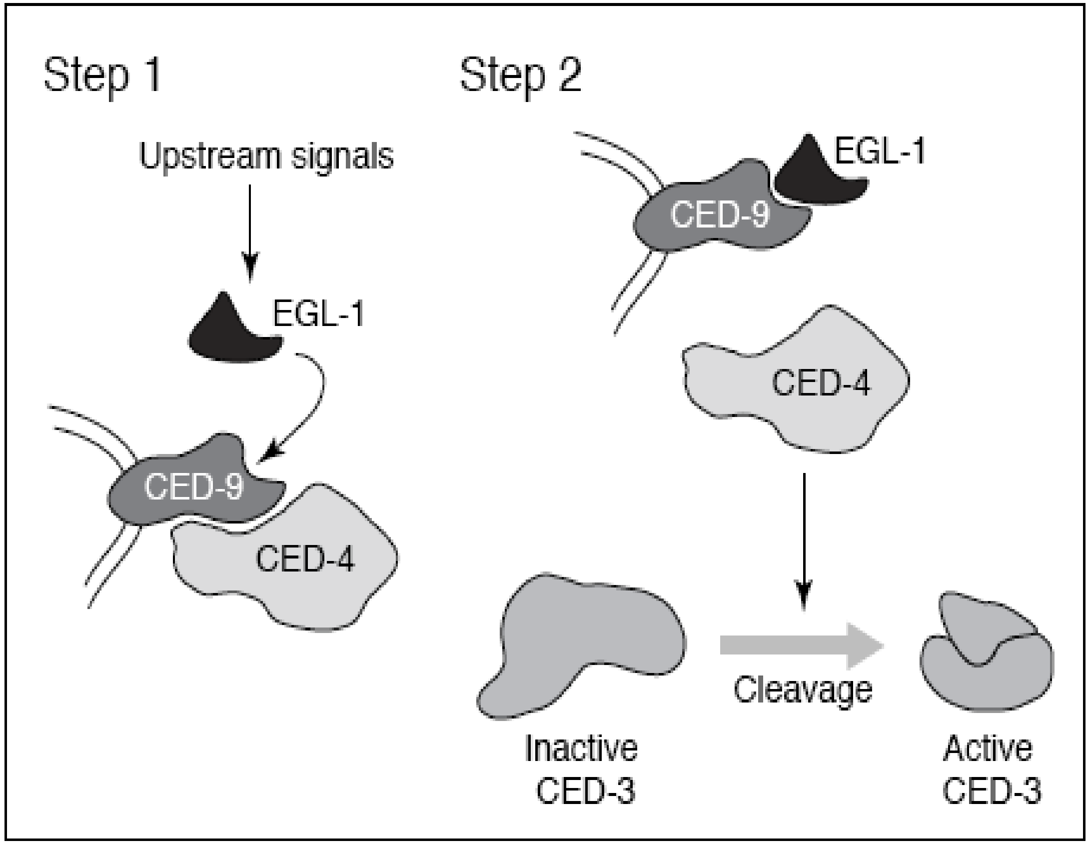
Genetics of programmed cell death in C. elegans: past, present and future
Trends In Genetics Oct 1998 Vol. 14 n.10
METZSTEIN, STANFIELD, HORVITZ

(lf) = loss-of-function mutation

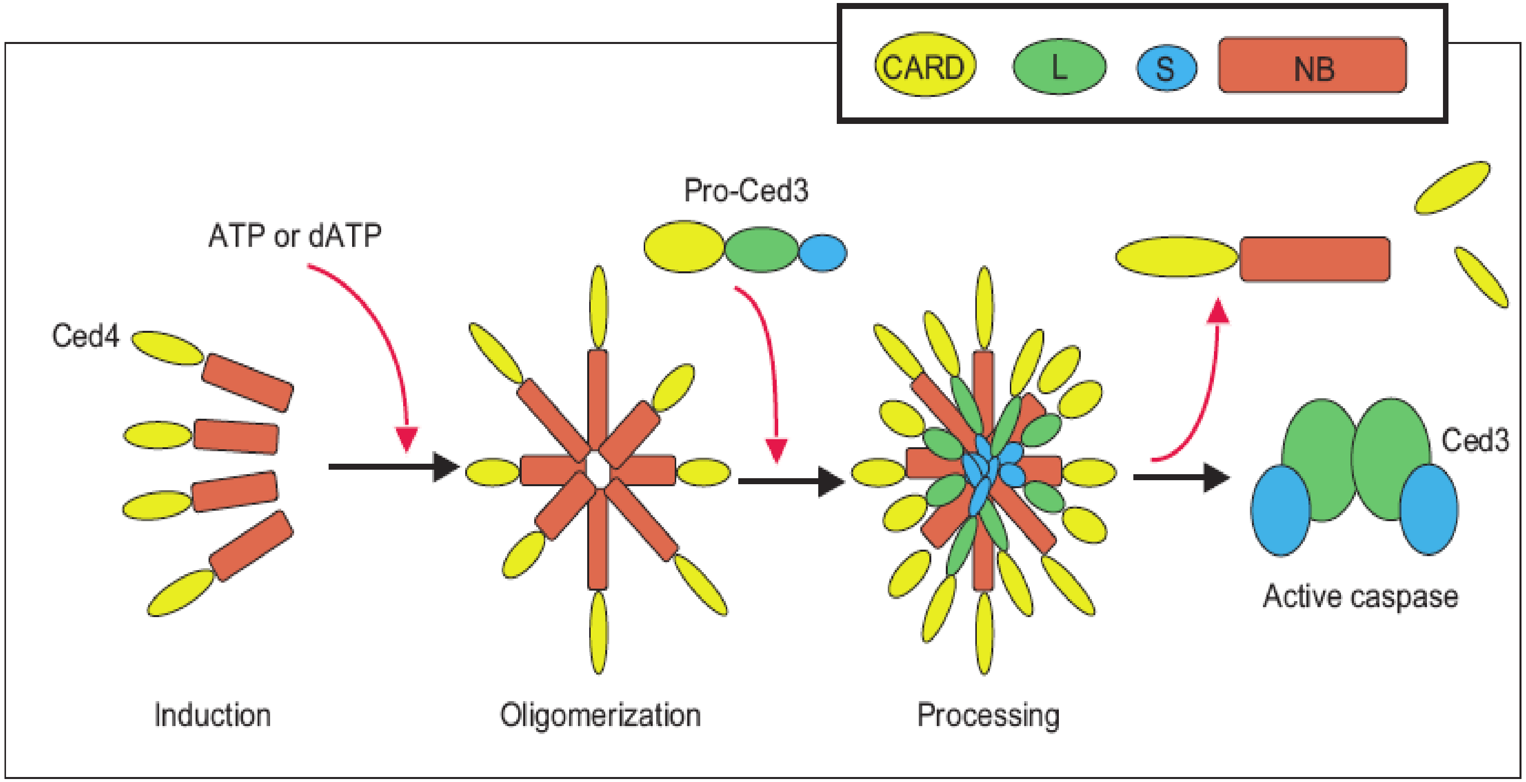
LIVE CELL



APOPTOSIS



Step 1
Upstream signals
EGL-1
CED-9
CED-4
Step 2
EGL-1
CED-9
CED-4
Inactive
CED-3
Cleavage
Active
CED-3



Science's STKE

Sci. STKE, Vol. 2004, Issue 239, pp. re9, 29 June 2004

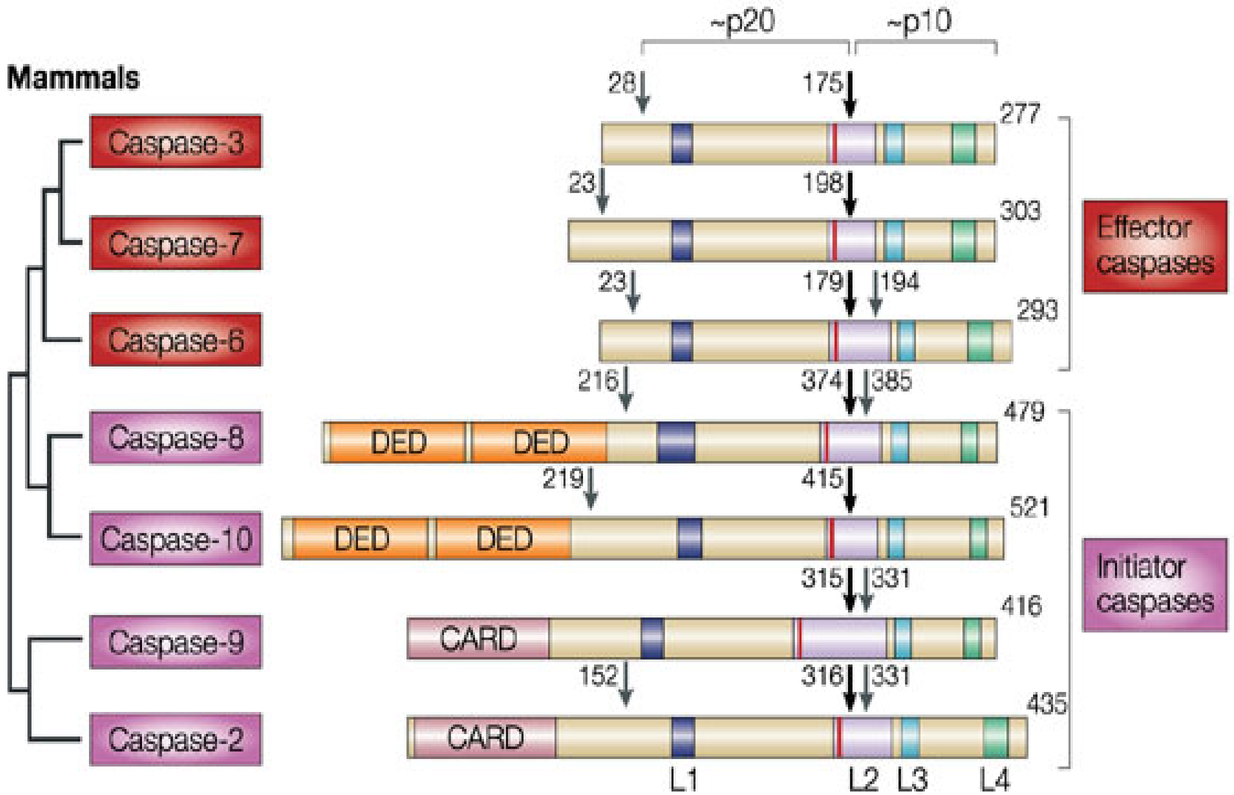
The Domains of Apoptosis: A Genomics Perspective

John C. Reed, Kutbuddin S. Doctor, and Adam Godzik

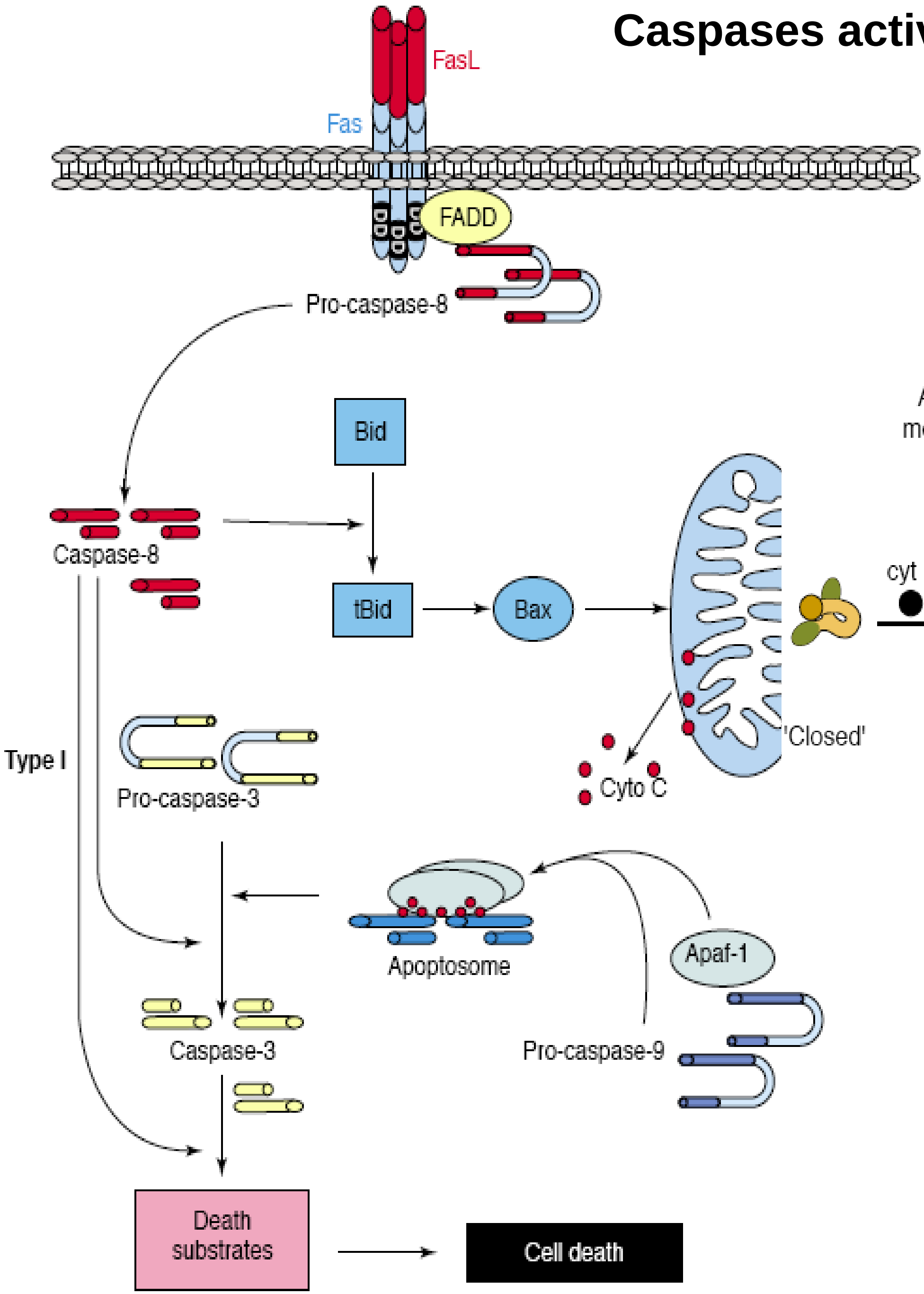
Cell death regulation in mammals

- Caspases
- APAF-1
- Bcl-2 family

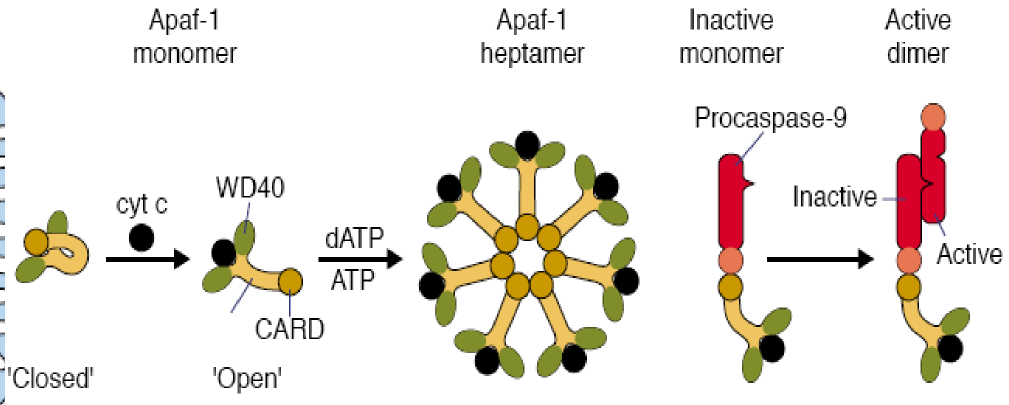
Caspases family



Caspases activation



Current Opinion in Pharmacology

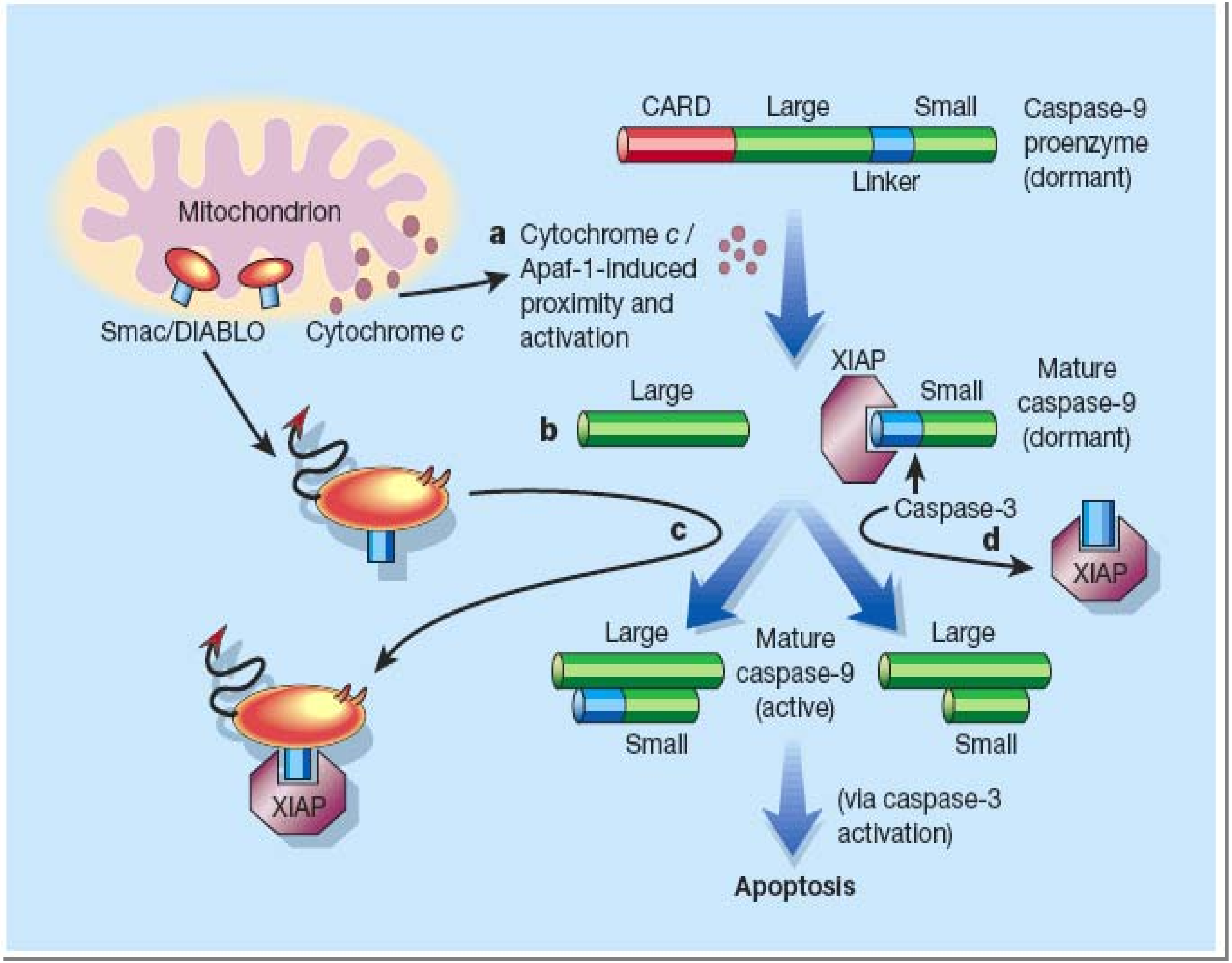


Current Opinion in Cell Biology

-Enzyme
- Inactive and pre-existing
- Activated during apoptosis
-Cascade
- DNA digestion
- Phagocytosis

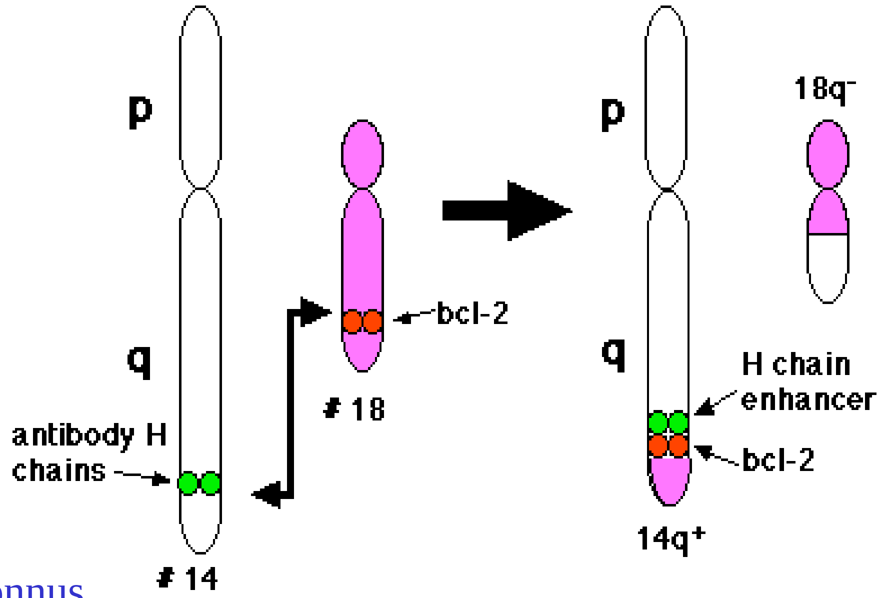
Caspases deficiency

Mouse	*caspase-1*	Yes – knockout [62,63]. (Animals develop normally.)	Defects in death-receptor-mediated apoptosis [63]	Defects in production of IL-1α and IL-1β [62,63]
	caspase-2	Yes – knockout [76]. (Animals have excess oocytes.)	Oocytes resistant to cell death [76]	None identified
	caspase-3	Yes – knockout [13]. (Perinatal lethality. Animals have excess brain tissue.)	Defects in brain apoptosis [13,77]	Skeletal muscle differentiation [78]
	caspase-6	Yes – knockout [79]. (Animals develop normally.)	Not determined	Not determined
	caspase-7	Yes – knockout [79]. (Embryonic lethality.)	Not determined	Not determined
	caspase-8	Yes – knockout [59]. (Embryonic lethality, impaired heart-muscle development and decreased pool of hematopoietic precursors.)	Defects in Fas and TNF-initiated cell death [59]	T-cell function [66]
	caspase-9	Yes – knockout [11,12]. (Perinatal lethality. Animals have excess brain tissue.)	Defects in brain apoptosis [11,12]. Defects in cell death in response to UV or γ-irradiation [12]	None identified
	caspase-11	Yes – knockout [64]. (Animals develop normally.)	Defects in oligodendrocyte-mediated cell death [67]	Defects in production of cytokines IL-1α and IL-1β [64]
	caspase-12	Yes – knockout [80]. (Animals develop normally.)	Fibroblasts are defective in cell death in response to endoplasmic reticulum stress stimuli [80]	None identified
Human	*caspase-8*	Yes – familial mutation [65].	Defects in death-receptor-mediated apoptosis [65]	Defects in activation of T, B and NK cells [65]
	caspase-10	Yes – familial mutation [14]. (Patients have autoimmune lymphoproliferative syndrome type II.)	Defects in death-receptor-mediated apoptosis [14]	None identified


CARD
Large
Small
Caspase-9 proenzyme (dormant)
Linker
Mitochondrion
Smac/DIABLO
Cytochrome c
a Cytochrome c / Apaf-1-induced proximity and activation
b
Large
XIAP
Small
Mature caspase-9 (dormant)
Caspase-3
c
d
XIAP
Large
Small
Mature caspase-9 (active)
Large
Small
XIAP
(via caspase-3 activation)
Apoptosis

Le gène Bcl-2

1985: identification de Bcl-2
(**B-cell leukemia / lymphoma 2-like protein)**
dans les lymphomes folliculaires B
ayant la translocation t(14;18)



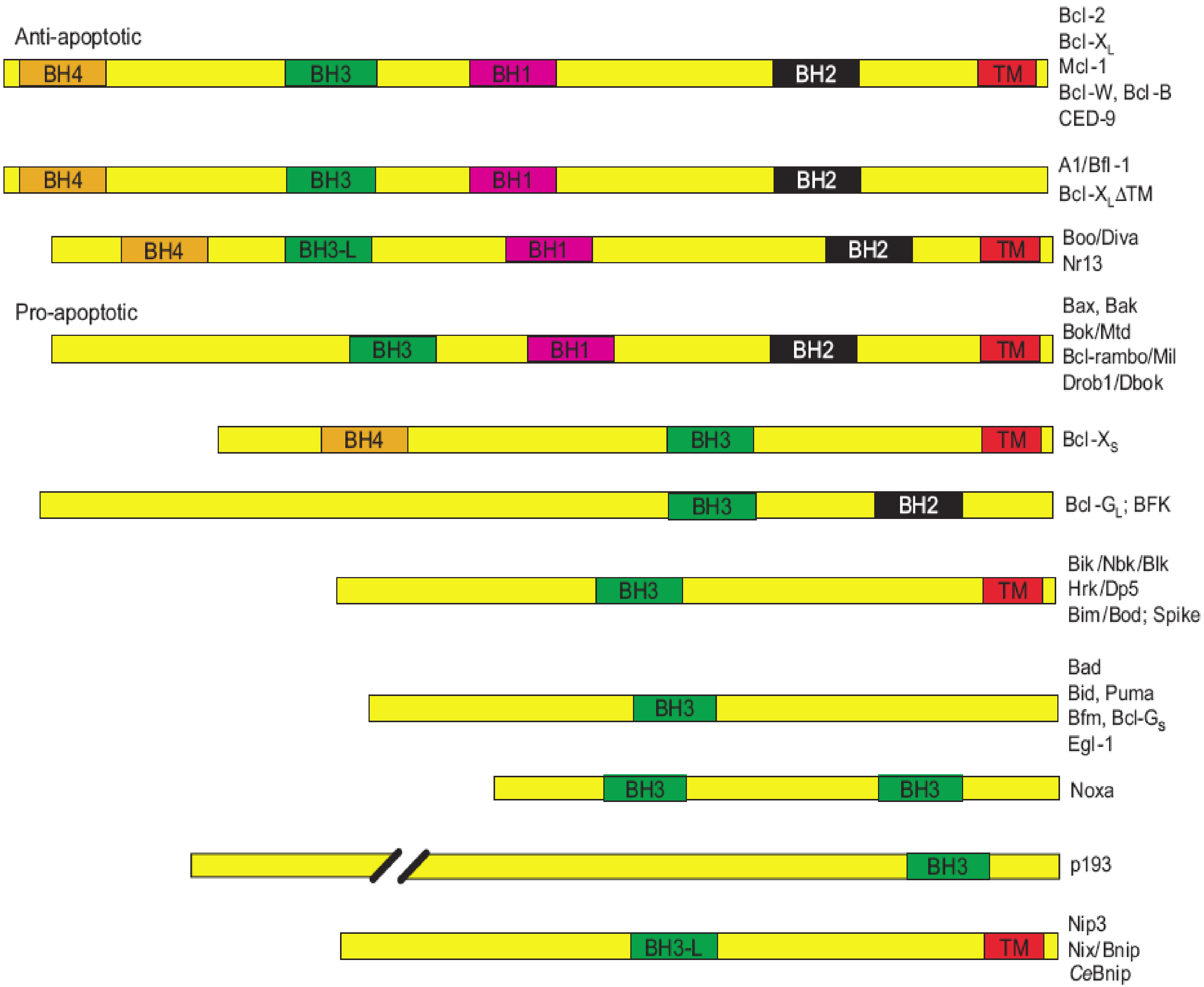
1988, 1989: contrairement aux autres oncogènes connus,
Bcl-2 n'induit pas la prolifération cellulaire mais bloque la mort cellulaire induite par de nombreux stimuli

1990: détection de son expression à la mitochondrie

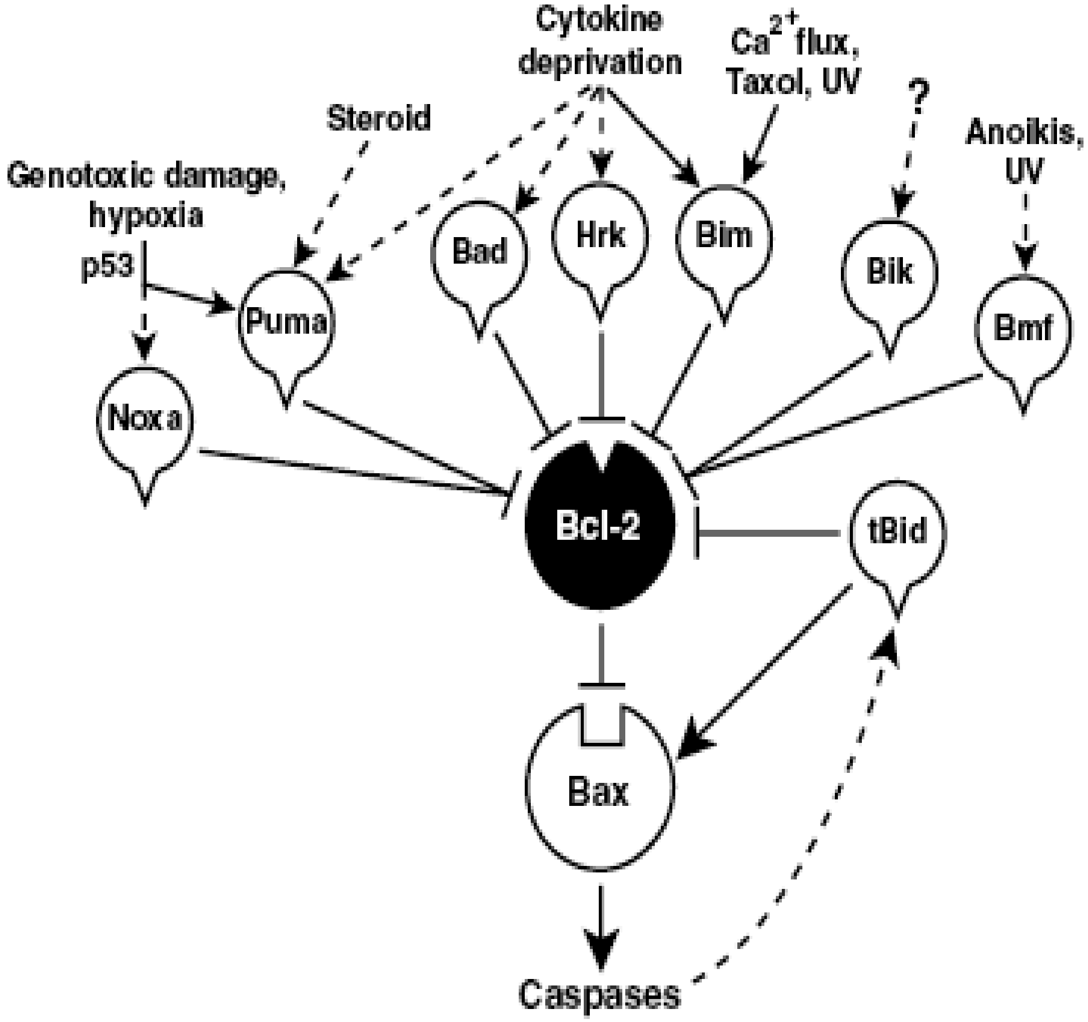
1989: les souris transgéniques Ig-Bcl-2 développent une hyperplasie folliculaire polyclonale de cellules B au repos puis des lymphomes monoclonaux (avec activation de c-myc)

1991: souris transgéniques bcl-2/c-myc développent des leucémies
$\Rightarrow$**synergie défaut apoptose + prolifération dérégulée**

1993: souris déficientes pour Bcl-2: apoptose thymocytes et mélanocytes

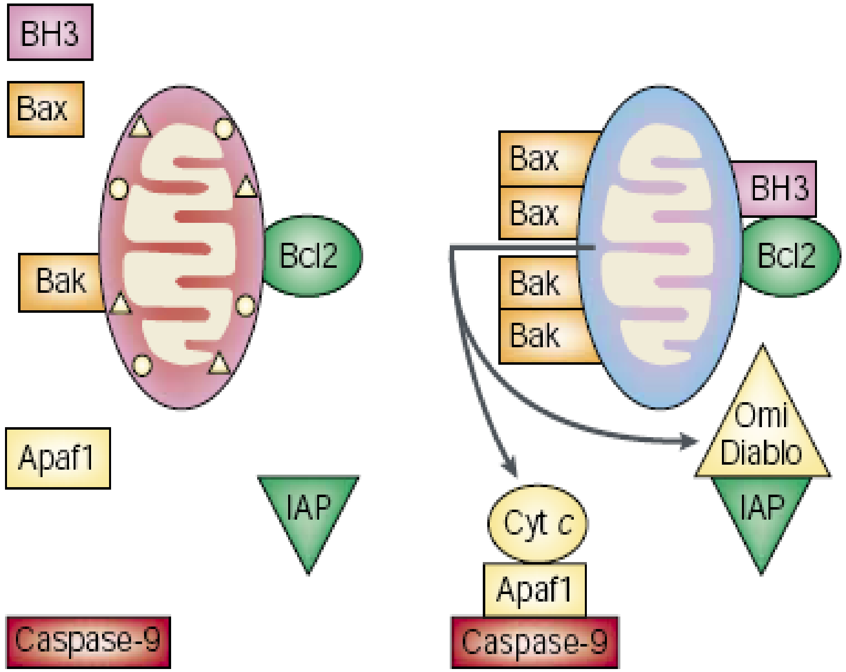

Anti-apoptotic
BH4
BH3
BH1
BH2
TM
Bcl-2
Bcl-X$_L$
Mcl-1
Bcl-W, Bcl-B
CED-9
A1/Bfl-1
Bcl-X$_L$ΔTM
BH3-L
Boo/Diva
Nr13
Pro-apoptotic
Bax, Bak
Bok/Mtd
Bcl-rambo/Mil
Drob1/Dbok
Bcl-X$_S$
Bcl-G$_L$; BFK
Bik/Nbk/Blk
Hrk/Dp5
Bim/Bod; Spike
Bad
Bid, Puma
Bfm, Bcl-G$_S$
Egl-1
Noxa
p193
Nip3
Nix/Bnip
*Ce*Bnip

BH3-only

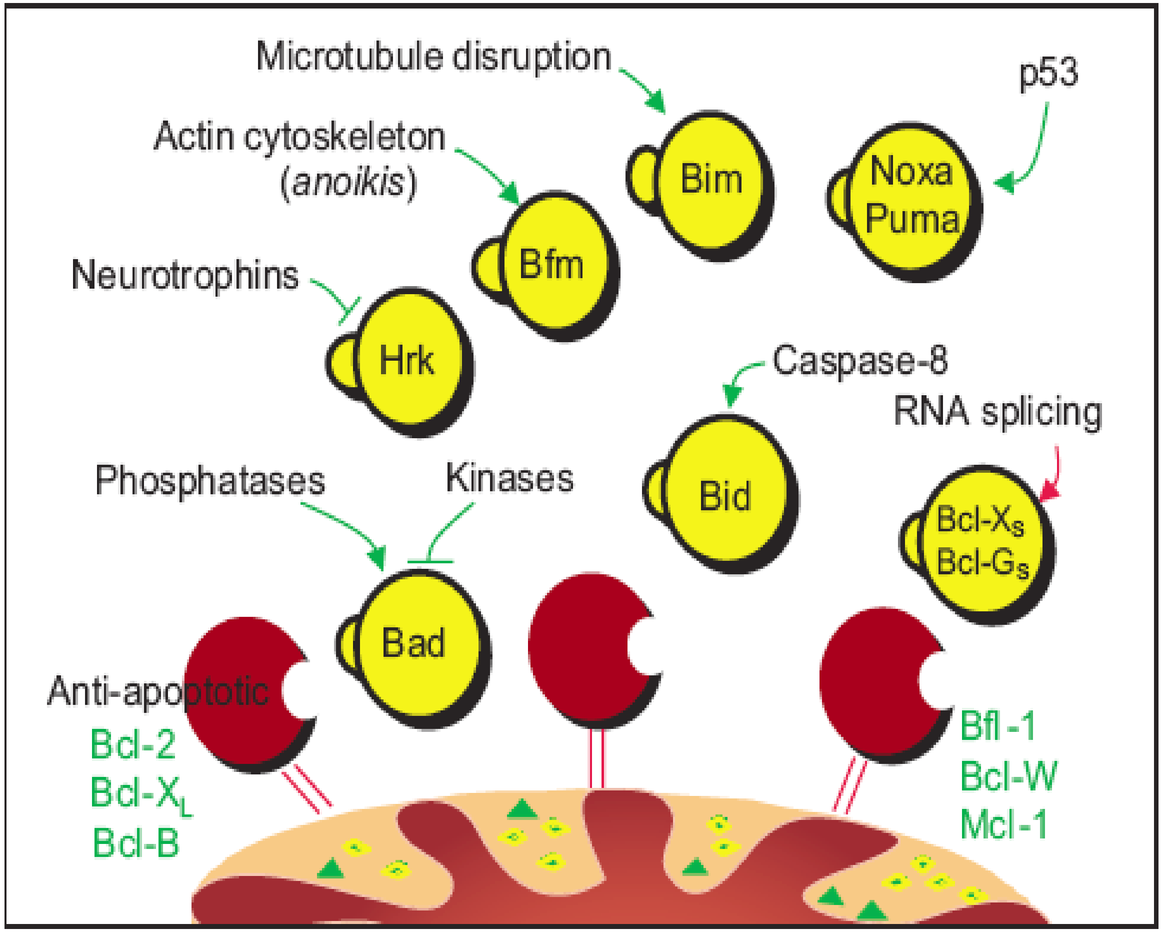


Cytokine deprivation
Ca2+ flux, Taxol, UV
Steroid
?
Anoikis, UV
Genotoxic damage, hypoxia
p53
Bad
Hrk
Bim
Bik
Bmf
Puma
Noxa
Bcl-2
tBid
Bax
Caspases

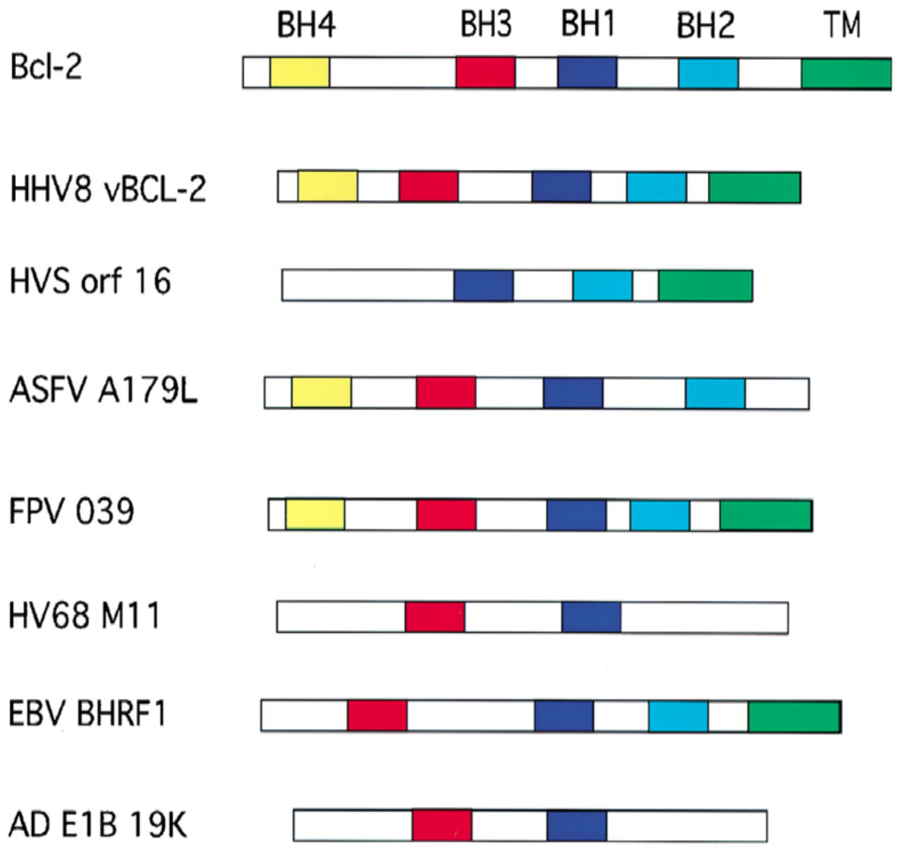
Apoptosis regulation by Bcl-2 family members



Bcl-2 regulation



Viral Bcl-2 homologs

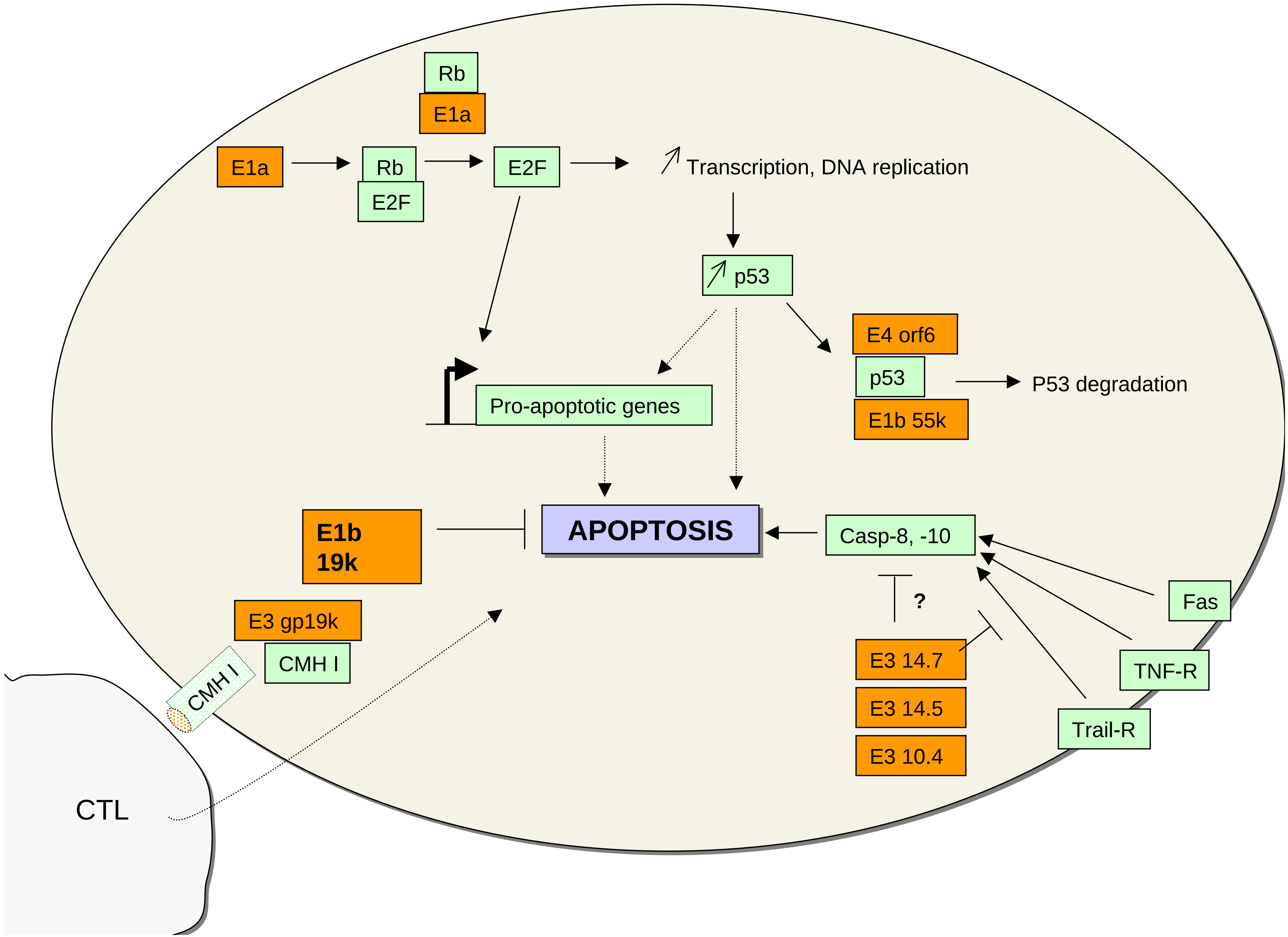


Andrea Cuconati et al. Genes Dev. 2002; 16: 2465-2478

Apoptosis regulation by adenoviruses

Adenovirus:
• Db strand DNA Virus à ADN db
• > 51 serotypes
• Infection tissus épithéliaux des voies respiratoires
• Responsables d’infections respiratoires: rhume -> pneumonie ou bronchite
• Escape from endosome to cytosol

• Transcription Adenovirus: 3 phases
- early (before virus replicaton): E1A, E1B 55kD, E1B 19kD, E2 (DNA synthesis), E3, E4 (DNA replication)
- intermediate and late (after virus replication): struct proteines and virions assembly
- cytolysis (>1000 virus particle/cell)

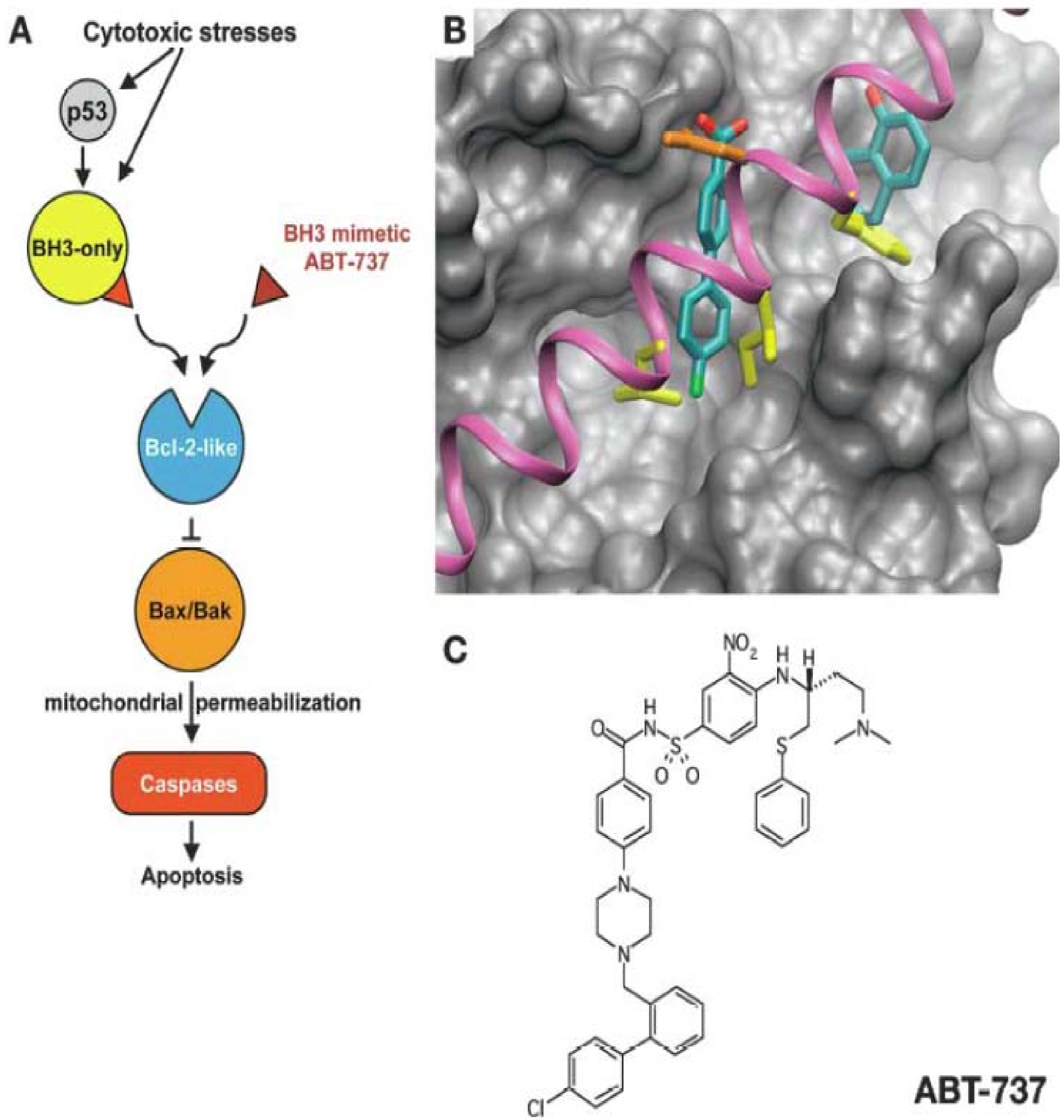

Rb
E1a
E1a
Rb
E2F
E2F
Transcription, DNA replication
p53
E4 orf6
p53
E1b 55k
P53 degradation
Pro-apoptotic genes
E1b 19k
APOPTOSIS
Casp-8, -10
?
Fas
TNF-R
Trail-R
E3 gp19k
CMH I
CMH I
E3 14.7
E3 14.5
E3 10.4
CTL

Vol 435|2 June 2005|doi:10.1038/nature03579 nature

LETTERS

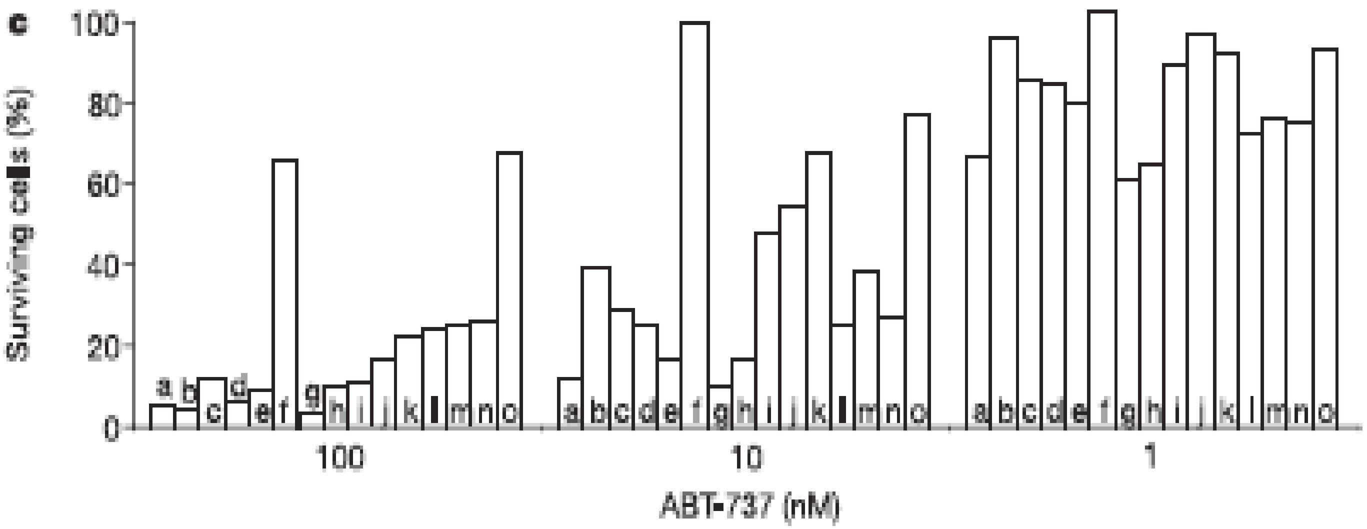
An inhibitor of Bcl-2 family proteins induces regression of solid tumours

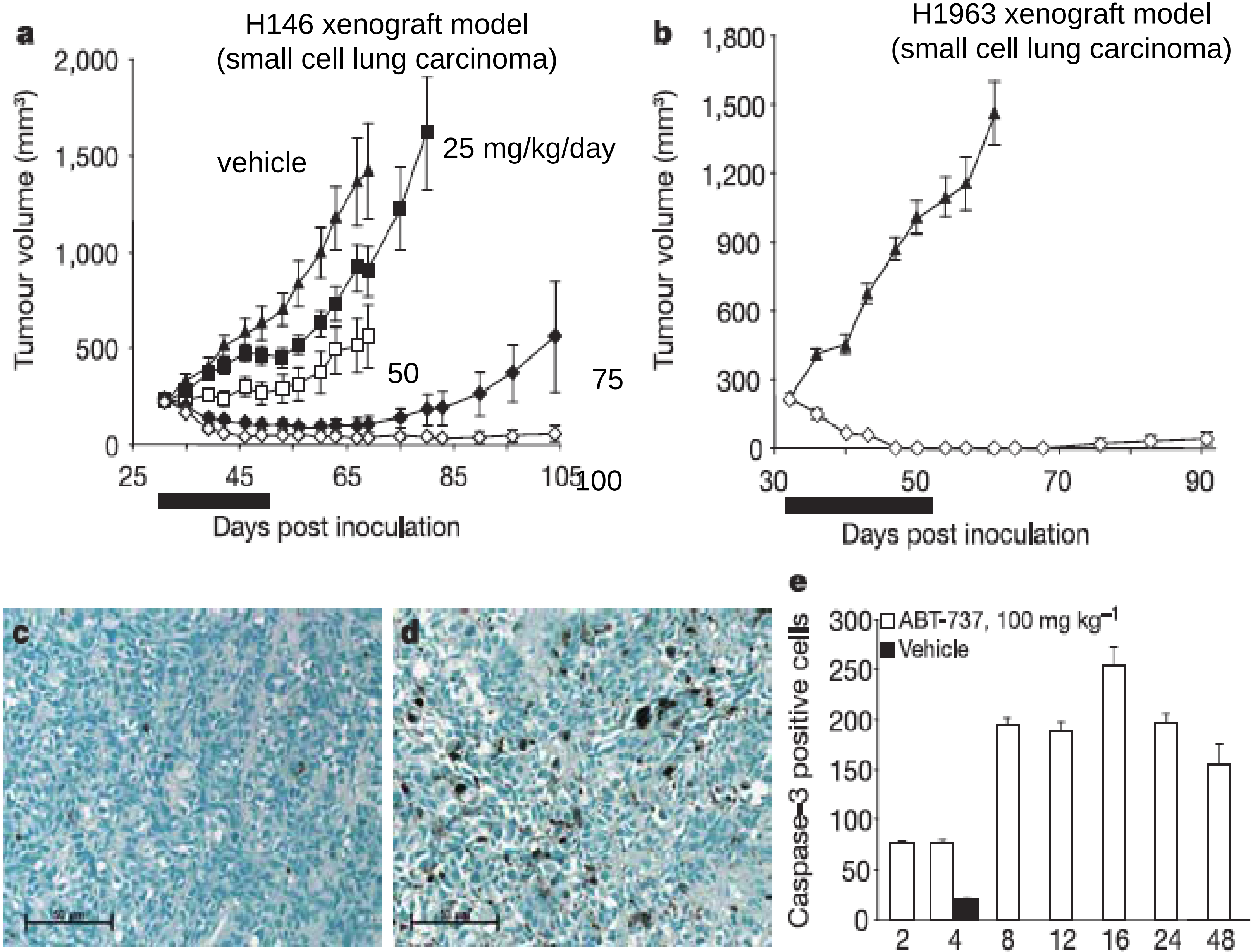
Tilman Oltersdorf[1]*, Steven W. Elmore[2]*, Alexander R. Shoemaker[2]*, Robert C. Armstrong[1], David J. Augeri[2], Barbara A. Belli[1], Milan Bruncko[2], Thomas L. Deckwerth[1], Jurgen Dinges[2], Philip J. Hajduk[2], Mary K. Joseph[2], Shinichi Kitada[3], Stanley J. Korsmeyer[4,5], Aaron R. Kunzer[2], Anthony Letai[5], Chi Li[6], Michael J. Mitten[2], David G. Nettesheim[2], ShiChung Ng[2], Paul M. Nimmer[2], Jacqueline M. O'Connor[2], Anatol Oleksijew[2], Andrew M. Petros[2], John C. Reed[3], Wang Shen[2], Stephen K. Tahir[2], Craig B. Thompson[6], Kevin J. Tomaselli[1], Baole Wang[2], Michael D. Wendt[2], Haichao Zhang[2], Stephen W. Fesik[2] & Saul H. Rosenberg[2]


A
Cytotoxic stresses
p53
BH3-only
BH3 mimetic
ABT-737
Bcl-2-like
Bax/Bak
mitochondrial permeabilization
Caspases
Apoptosis
B
C
ABT-737

Primary Chronic Lymphocytic Leukaemia cells:

% viabilité:

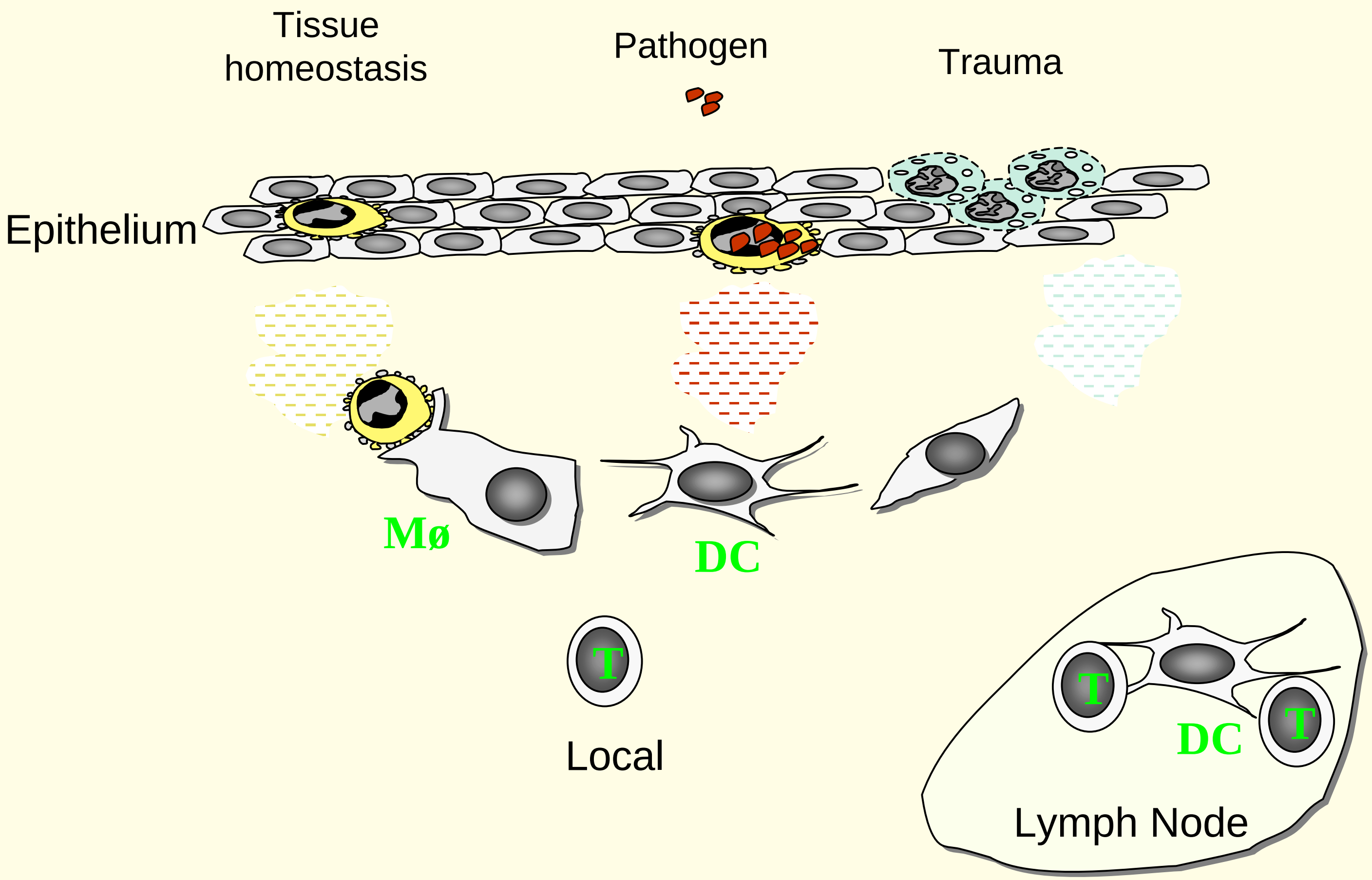




No treated ABT-737

Caspase-3 H146 xenograft model

Phagocytosis of dead cells: mechanisms and immune responses



I - Type of cell death

II- Phagocytosis of dead cells
= source of antigen

III- Antigen presentation + cytokine production

Inflammation resolution

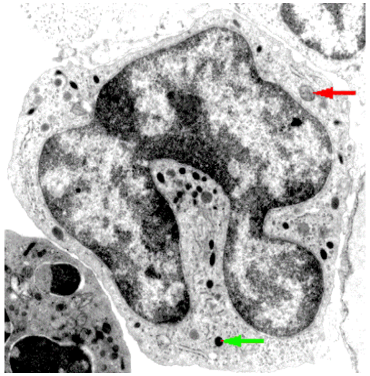
T cell activation (Immune response / tolerance)

Inflammation

Defect in dead cell phagocytosis and inflammation

Anti-tumoral responses

Macrophages



Bone marrow-derived:
monocytes in blood become macrophages in tissues

Found in all tissues:
- skin (Histiocytes),
- liver (Kupffer cells),
- lung (alveolar macrophages)
- spleen, lymph nodes, peritoneum
- central nervous system (microglia)
- Express phagocytic receptors

Heterogeneous population (various properties in different tissues)

Migration

Phagocytosis

Secretory function (enzymes that affect connective tissues, complement proteins, growth factors, chemokines, inhibitory interleukines, factors that promote tissue repair)

Microbicidal

Maturation (i.e. activation)

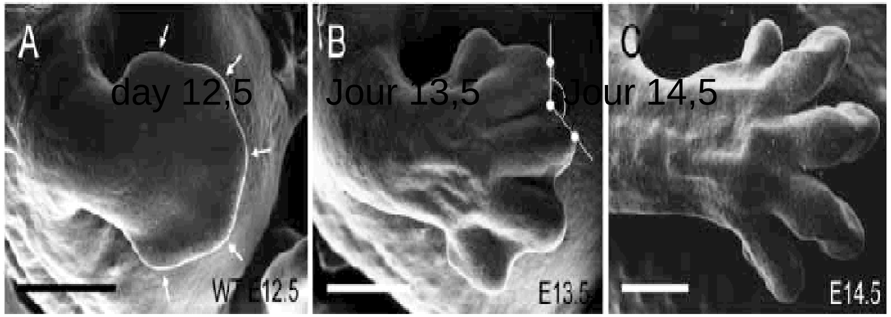
Antigen presentation (Class I and class II)

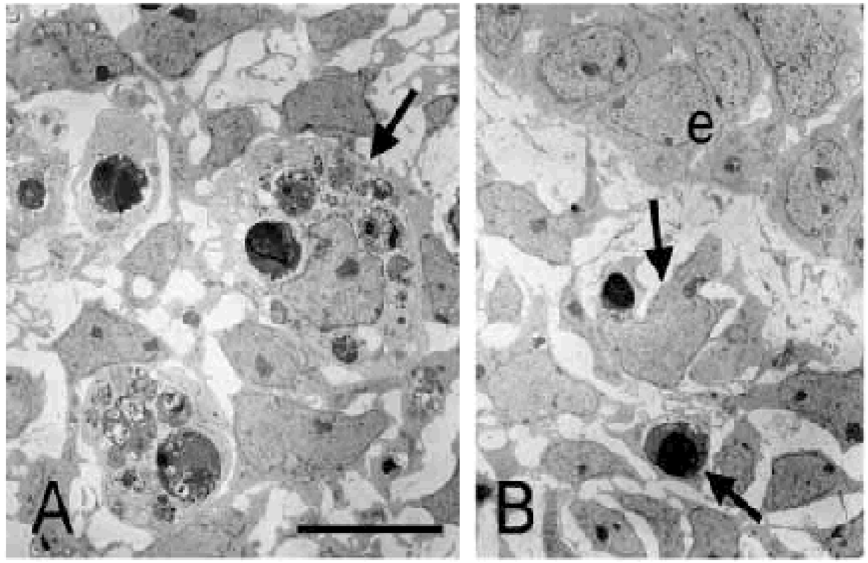
Phagocytosis of dead cells *in vivo* by macrophages

• Apoptotic T cells during selection in the thymus (Nature, 1994)

• Apoptotic interdigital cells (Wood, dev. 2000)

+/+





• Apoptotic hepatocytes (following intoxication with heavy metal) are phagocytosed by kupffer cells (Microscopy Research and Technique, 2002)

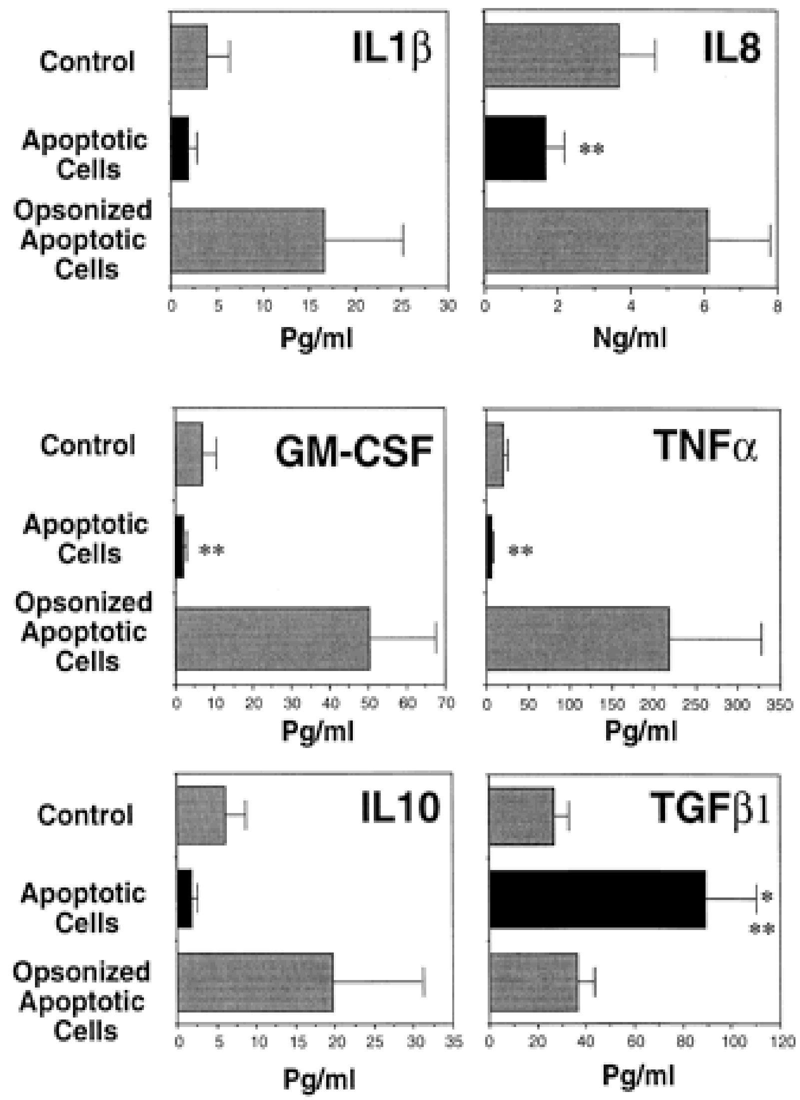
Apoptotic cell phagocytosis by macrophages induces their secretion of TGFβ1

Experimental system:

Mø -> supernatant->ELISA

Mø+apoptotic cells -> supernatant->ELISA

Mø+IgG opsonised apo cell -> supernatant->ELISA



Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM. J Clin Invest. 1998 Feb 15;101(4):890-8.

Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF.

Dendritic cells

Bone-marrow derived

Heterogeneity of dendritic celll subsets:
(myeloid and lymphoid): humans
- skin and stratified epithelium
(Langerhans cells)
- dermis
- splenic marginal
- germinal-centers DC
- thymus

Migration

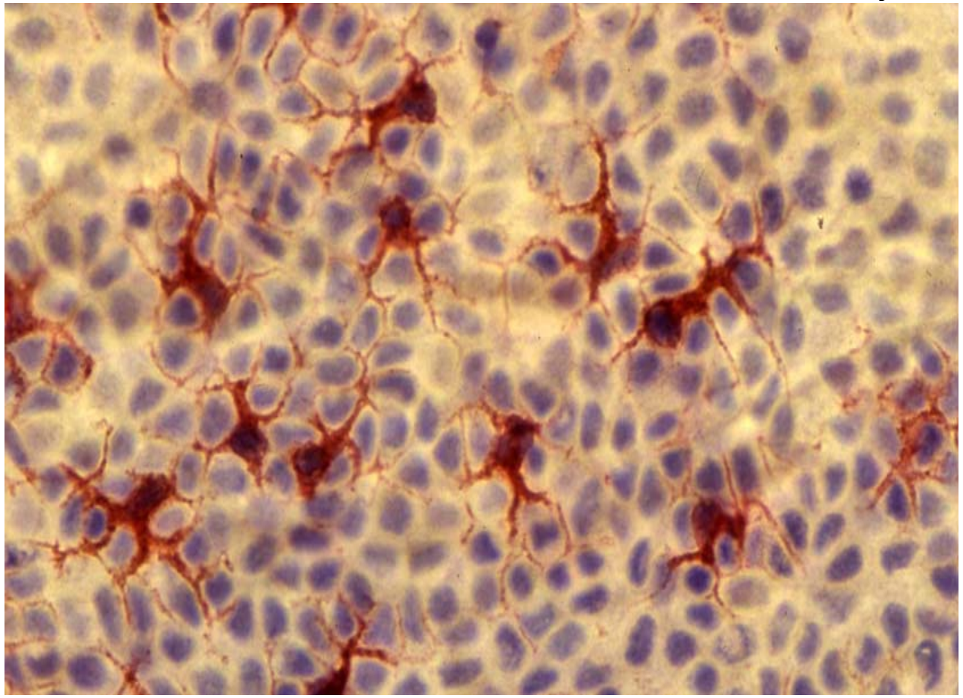
Endocytosis

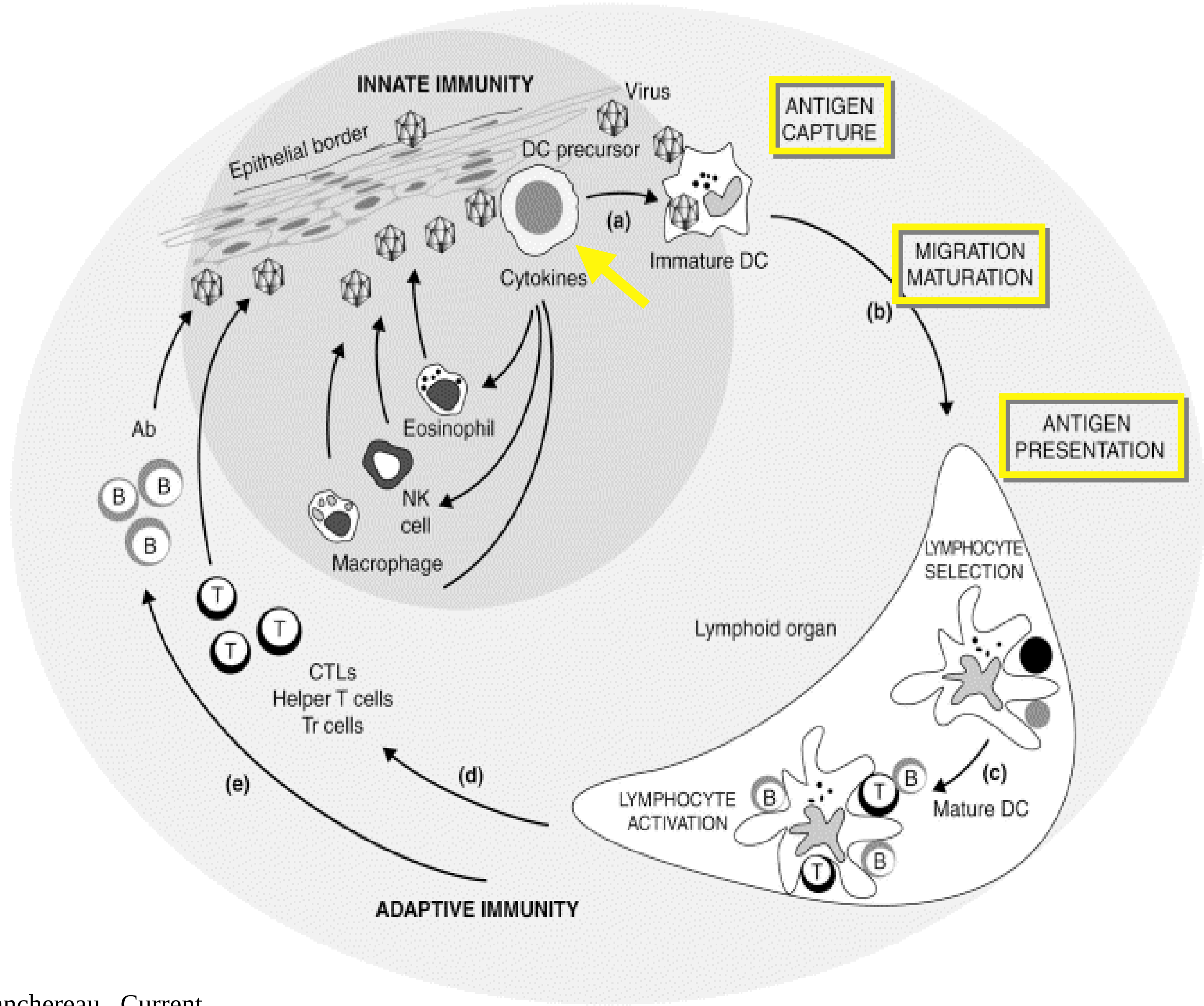
Maturation. Co-stimulatory molecules

Secretory function

Antigen presentation (Class I and
Class II). Cross presentation

anti-CMH-II, skin,
Pr. J-F. Nicolas, Lyon





Palucka and Banchereau , Current Opinion in Immunology, 2002, Pages 420-431

Current Opinion in Immunology

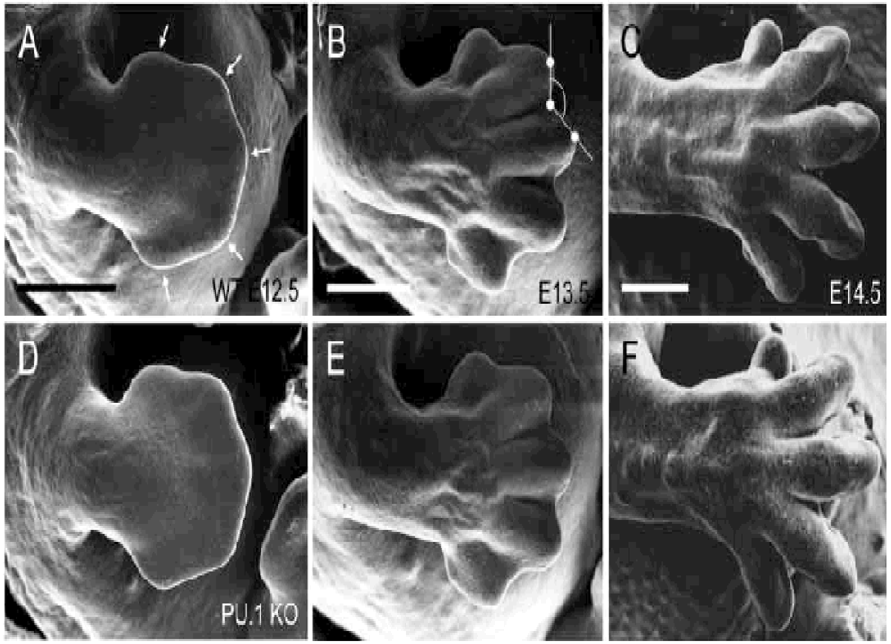
Other phagocytes implicated in apoptotic cell phagocytosis

In the absence of professional phagocytes (i.e. mice without macrophages, PU1-/-), apoptotic cells are phagocytosed by non professional phagocytes (Wood, dev. 2000):

day 12,5 Jour 13,5 Jour 14,5

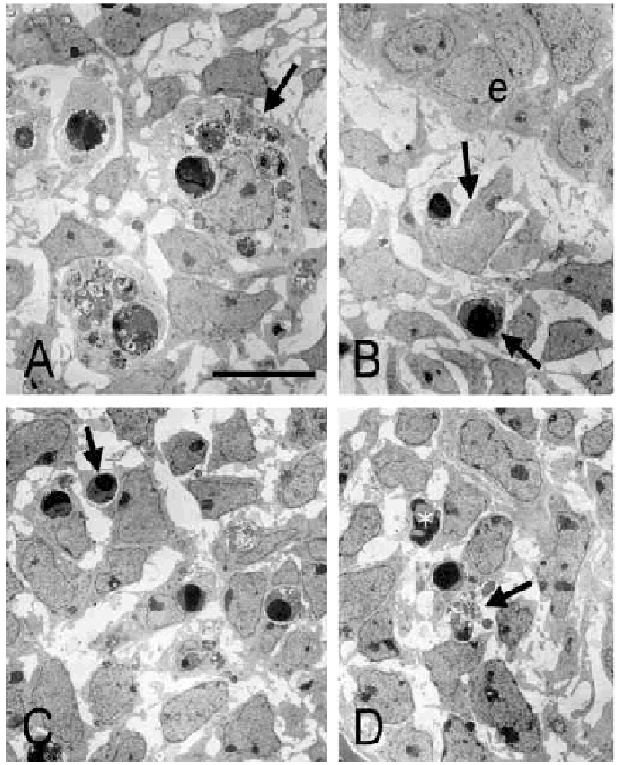
+/+

PU1-/-

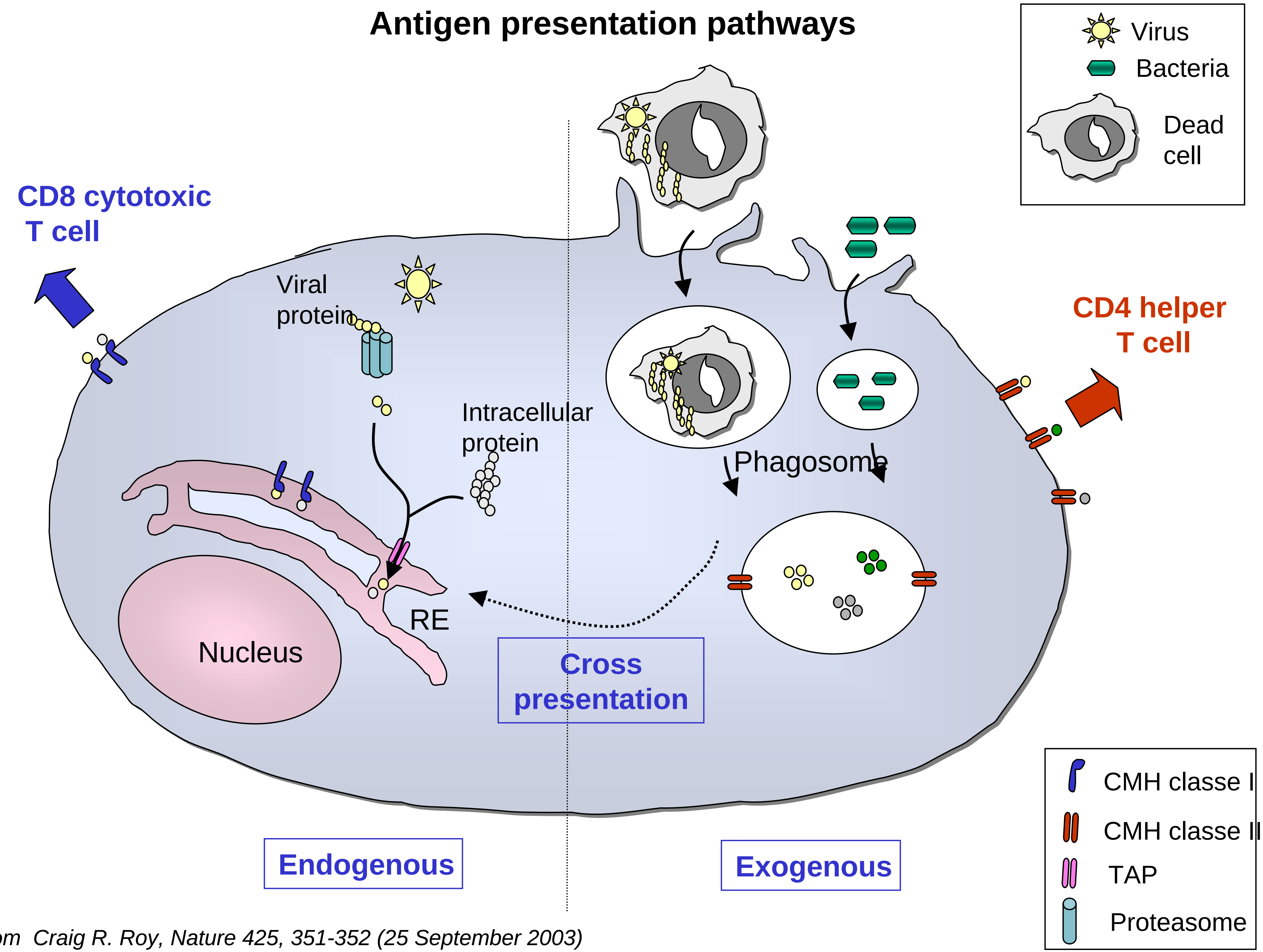


Apoptotic cell in macrophage

PU1-/- mice: Apoptotic cells in mesenchymal cells

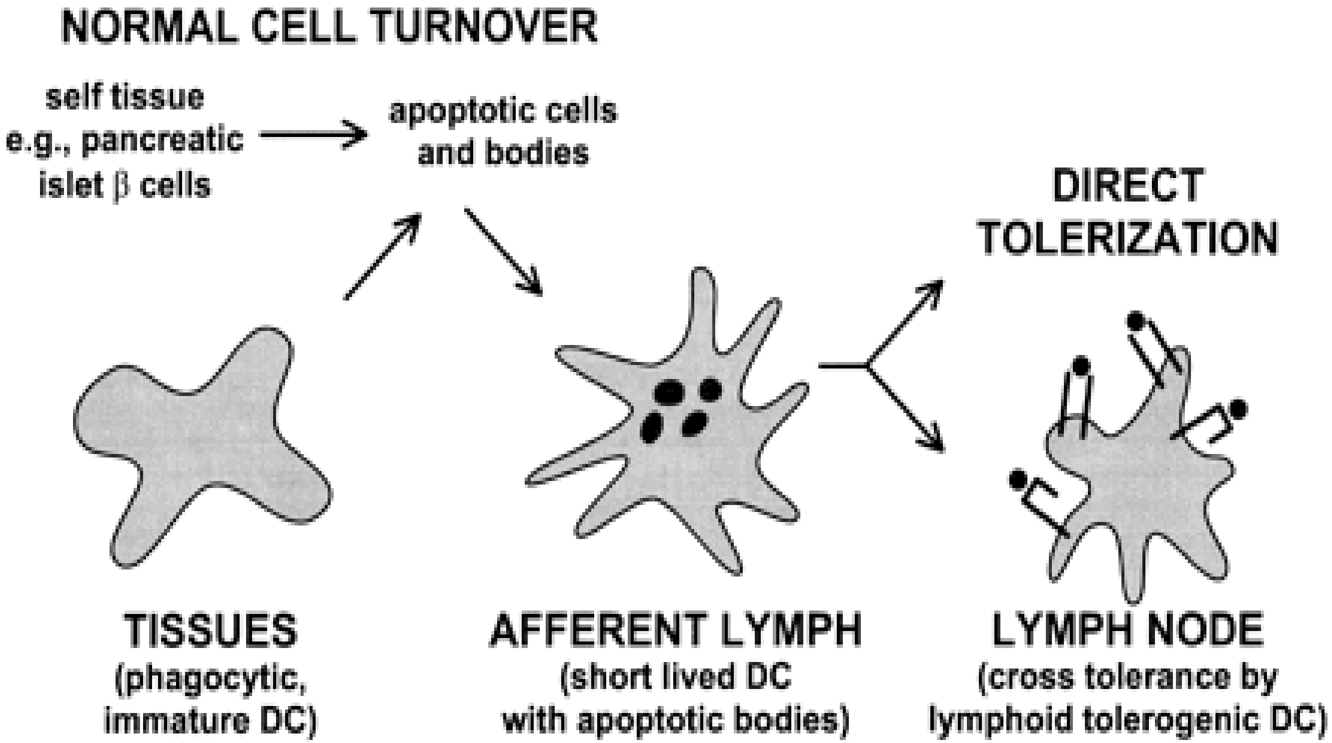


Antigen presentation pathways



from Craig R. Roy, Nature 425, 351-352 (25 September 2003)

Apoptotic cells can promote tolerance

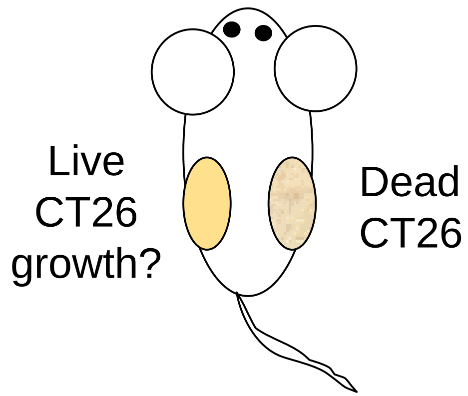


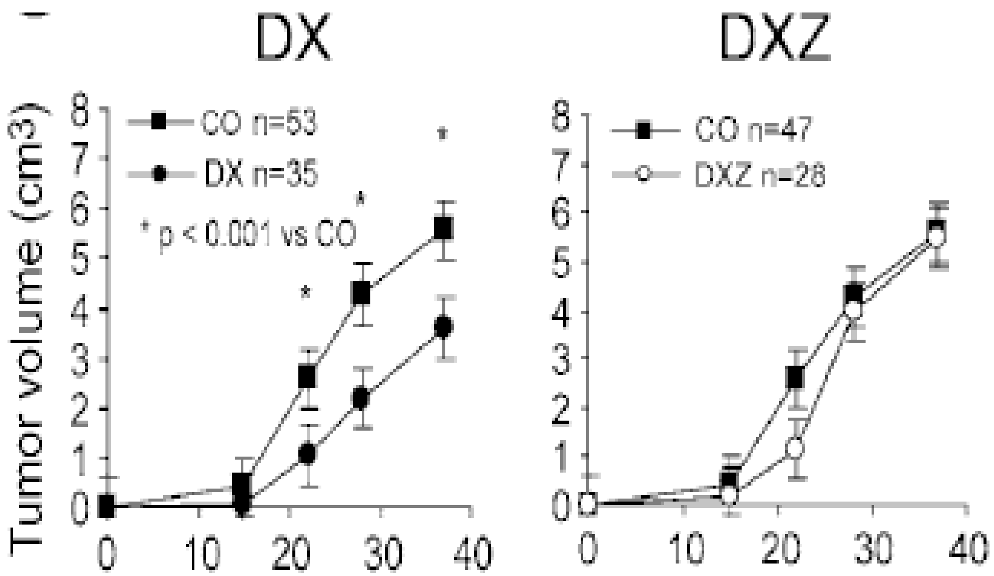
Steinman, J Exp Med. 2000

Apoptotic cells can also be immunogenic

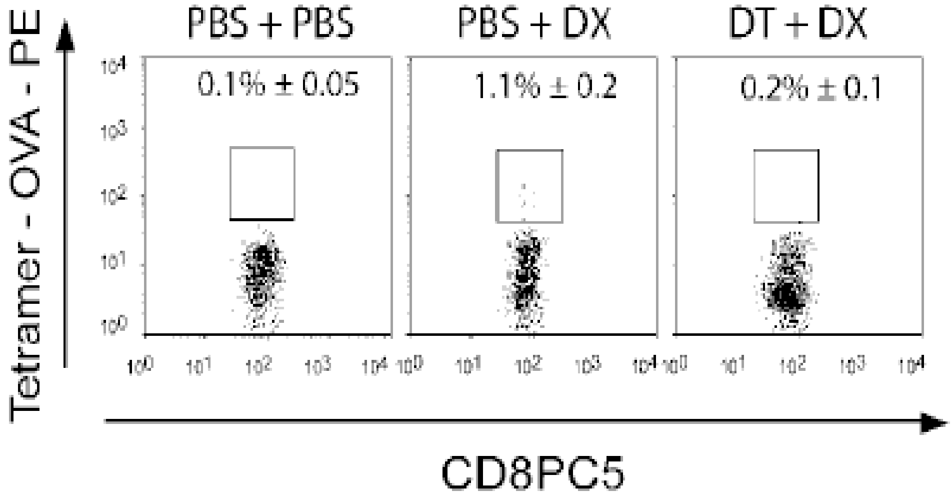
Caspase-dependent immunogenicity of doxorubicin-induced tumor cell death
Casares, J. Exp. Med, December 2005

- CT26 (colon carcinoma cell line)
  DX= doxorubicin
  DXZ= doxorubicin + ZVAD





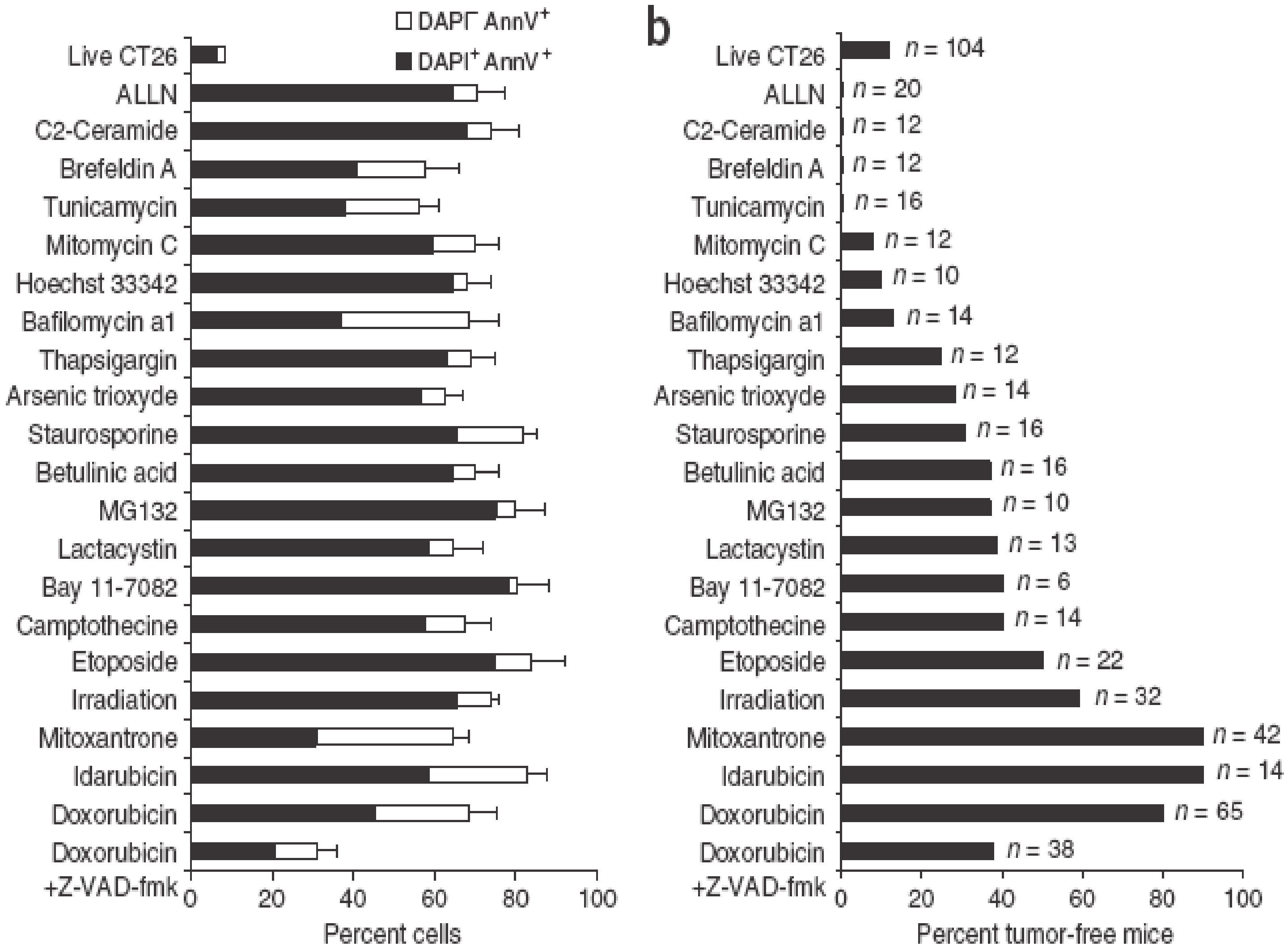
Apoptotic cells immunogenicity requires dendritic cells



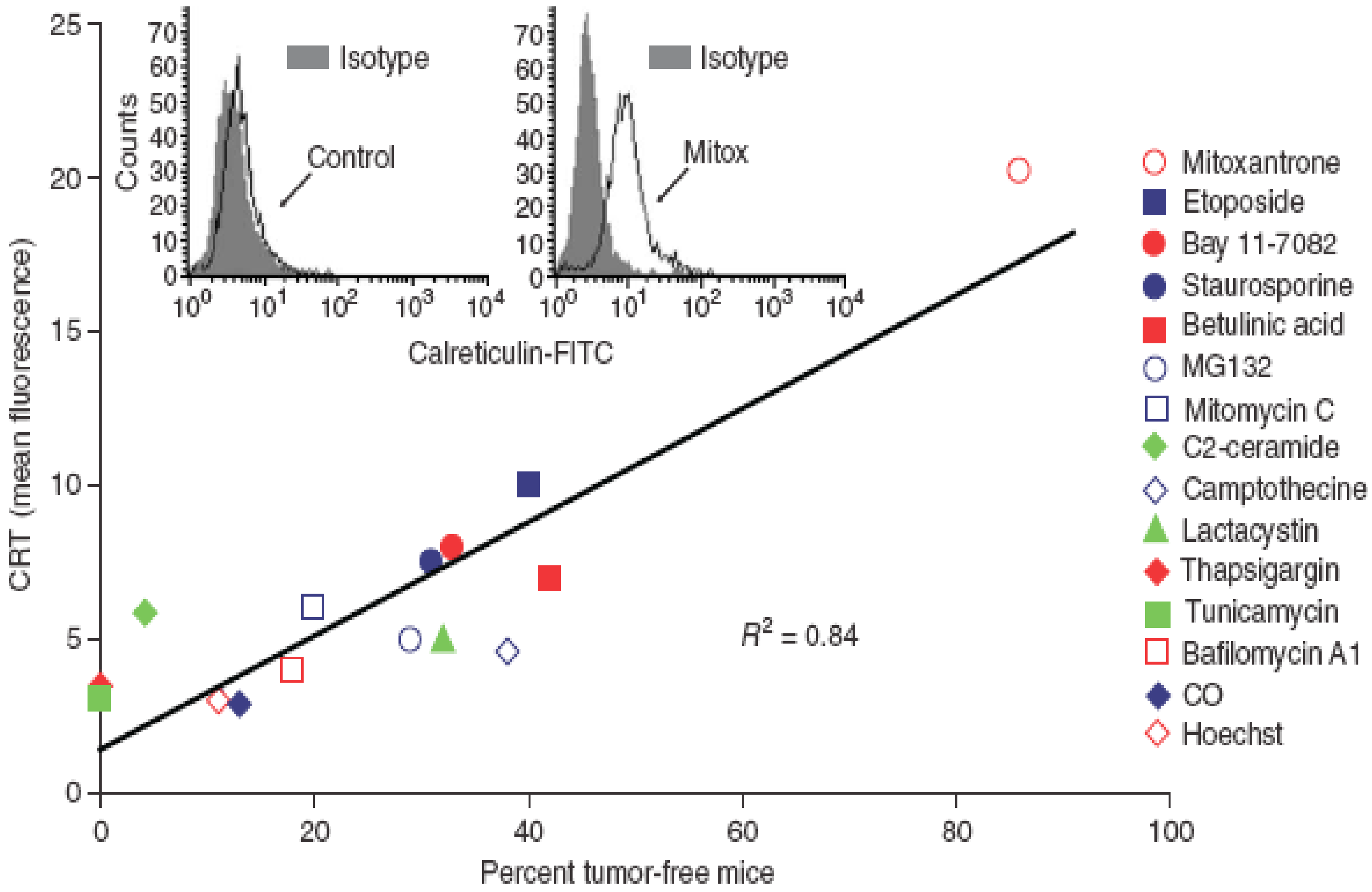
- Transgenic mice expressing diphteria toxin (DT) receptor in DCs pretreated with PBS alone or a dose of diphteria toxin that depletes DCs.
- The mice were then challenged with DX-treated B16-OVA cells into the food pad.
- Draining lymph nodes were recovered 48 h later and stained for simultaneous detection of CD8 and OVA peptide–specific T cell receptors.

Signal for immunogenicity?

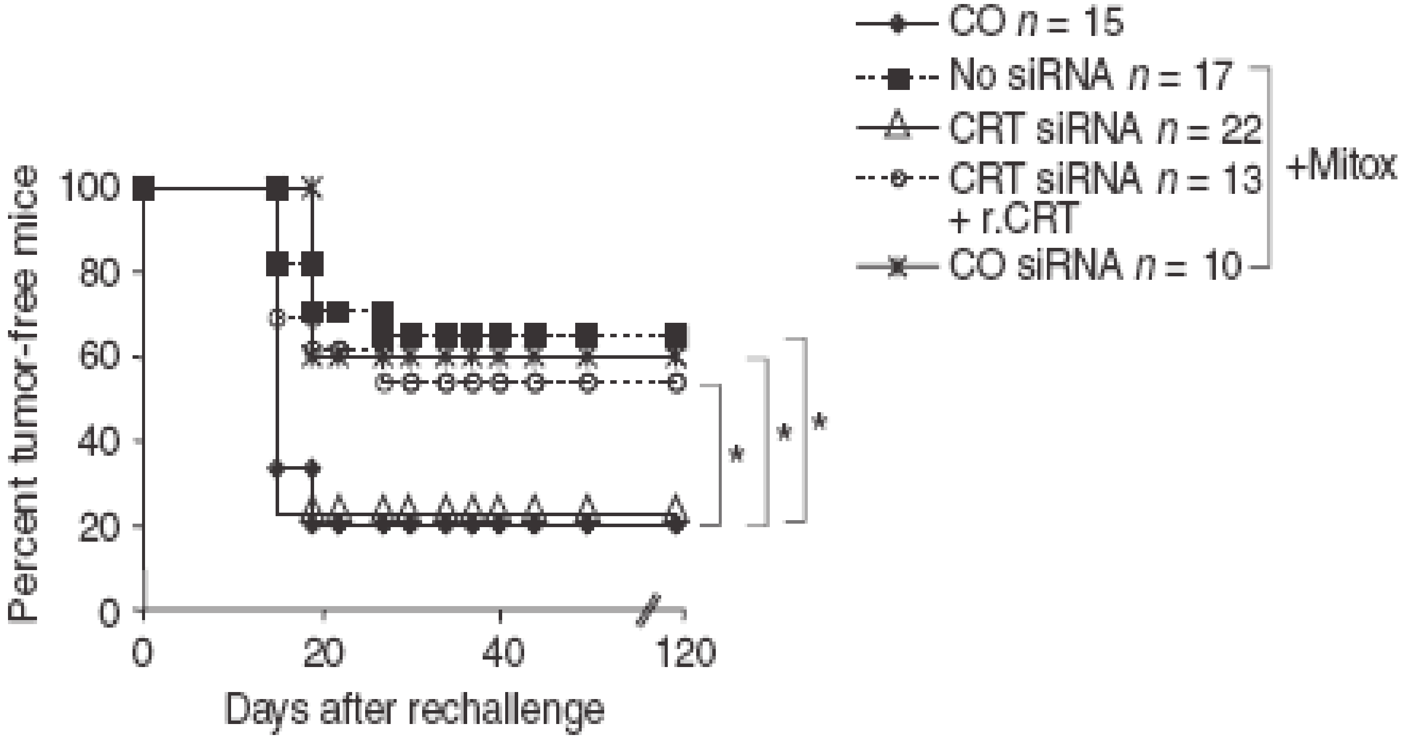
Obeid, Nat Med. 2007 Jan;13(1):54-61. Epub 2006 Dec 24.p



Calreticulin exposure dictates the immunogenicity of cancer cell death.

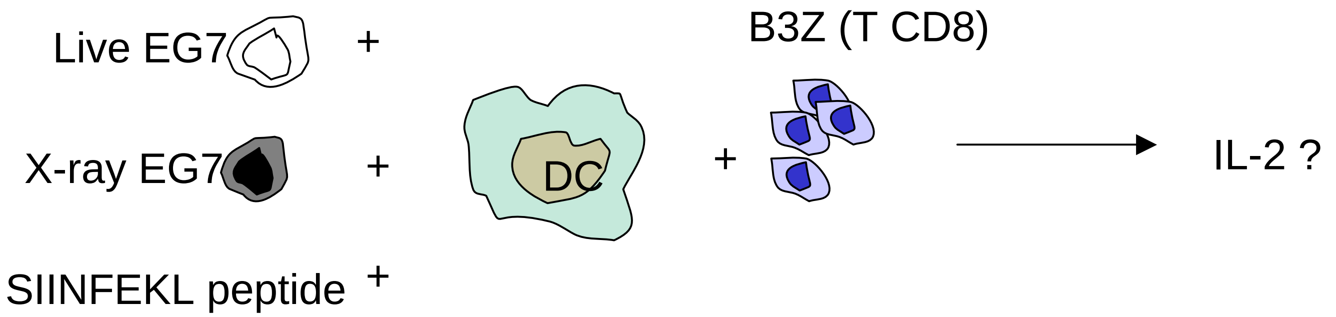


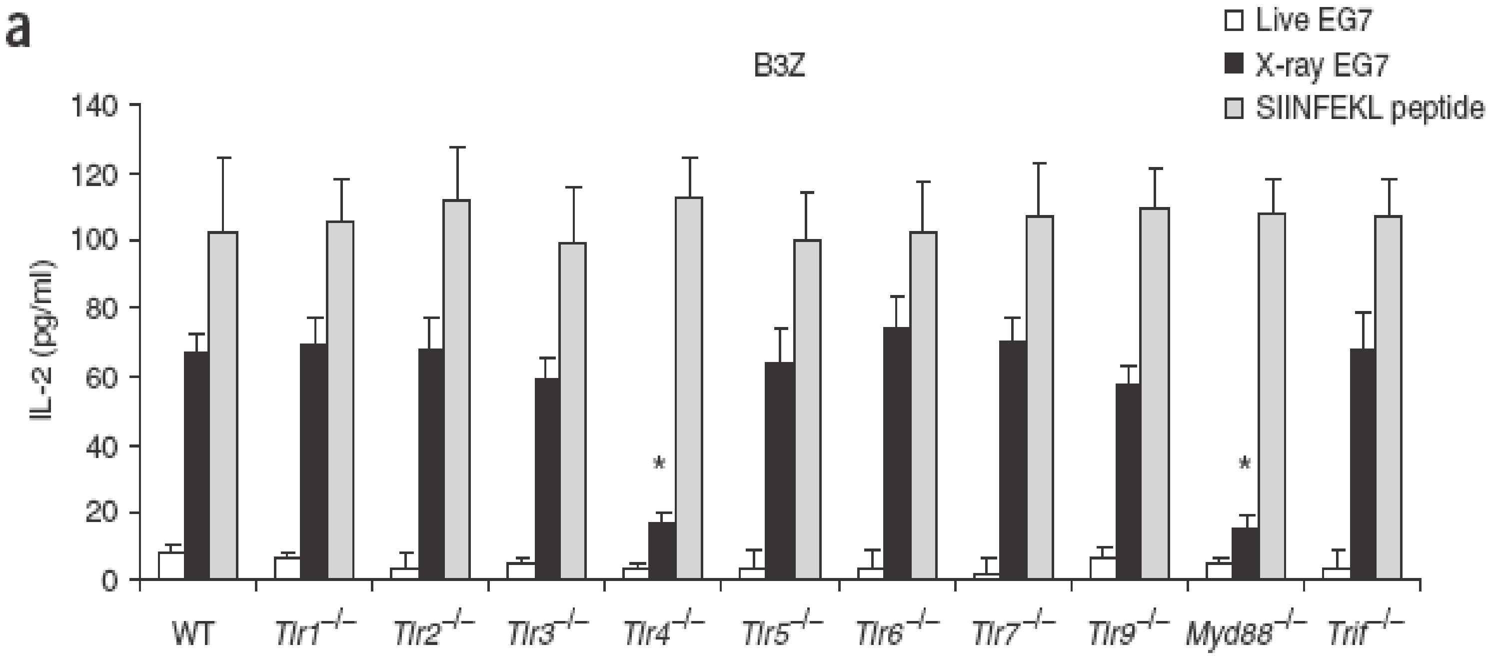
Calreticulin is required for the immune response against dying tumor cells



TLR4 controls antigen presentation by DCs engulfing apoptotic bodies in vitro.

EG7: OVA expressing cell line

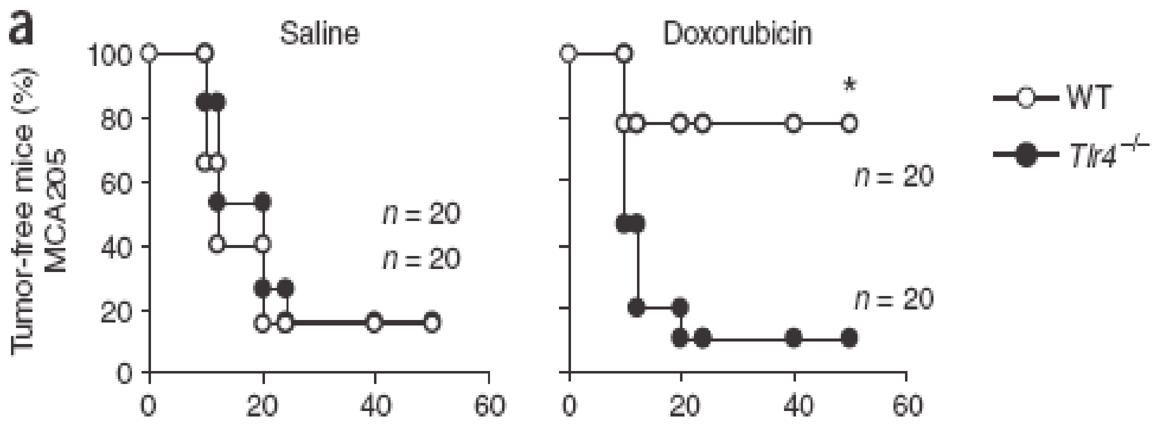




Apetoh, Nat Med. 2007 Sep;13(9):1050-9.

Inhibition of HMGB1 prevents the efficacy of vaccination with dying tumor cells.

• Mice (WT or Tlr4–/–) were immunized with PBS or dying tumor cells MCA205)and treated with doxorubicin.
At day 7: inoculation live tumor cells: monitoring tumor growth



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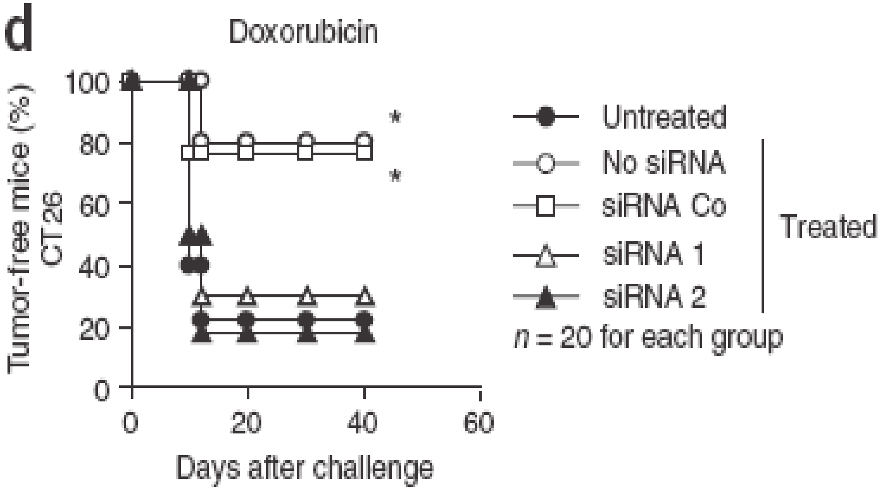


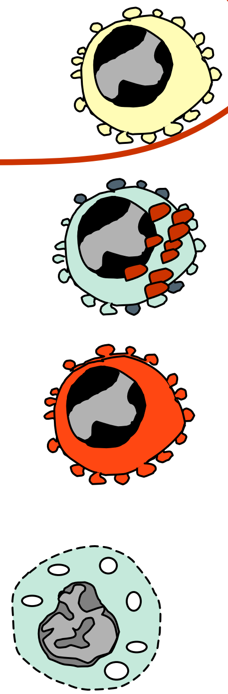
Form of cell death

Apoptosis 1

Apoptosis 2

Apoptosis 3

Necrosis



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décompresseur TIFF (non compressé)
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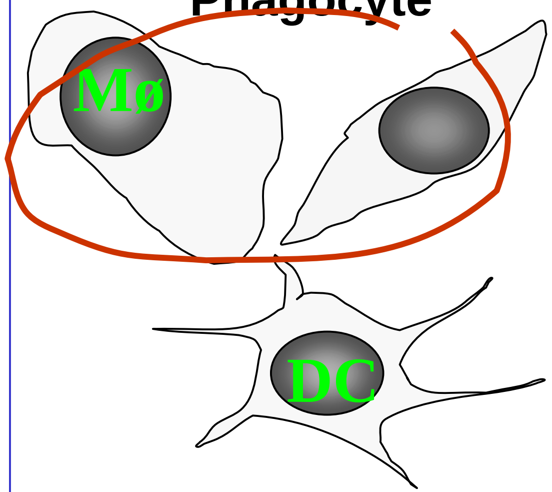
« danger signals » released ?

Phagocytic receptor(s)?

Phagocyte



Immunological outcomes

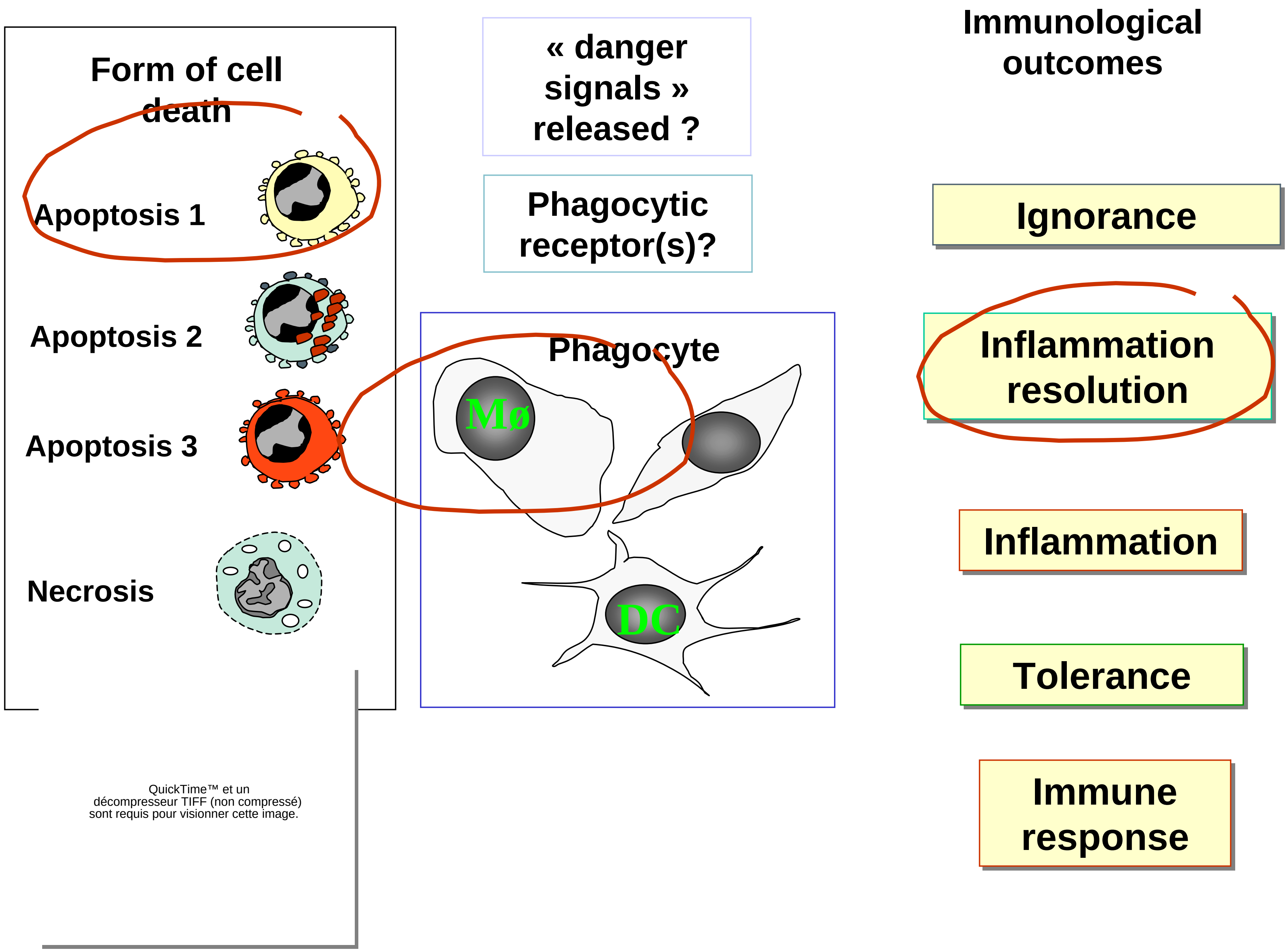
Ignorance

Inflammation resolution

Inflammation

Tolerance

Immune response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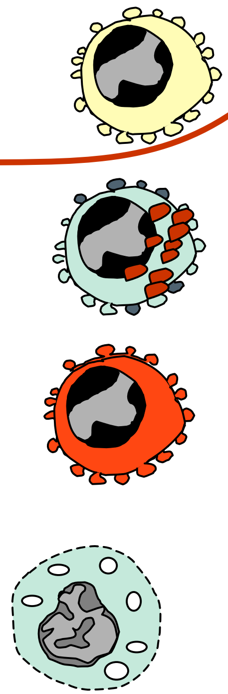

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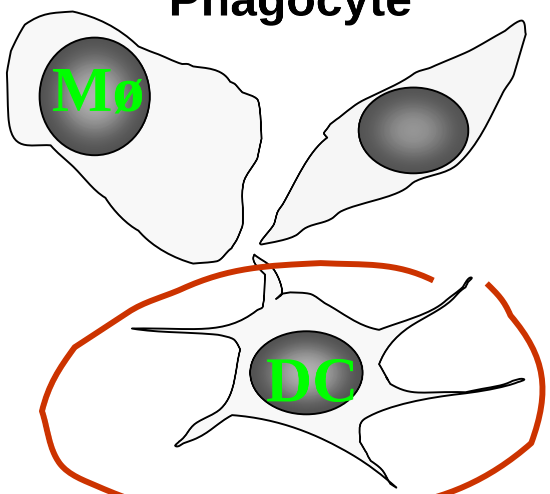

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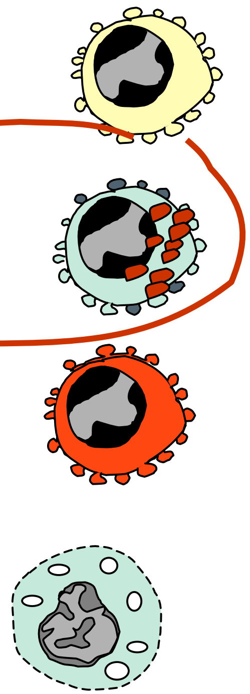

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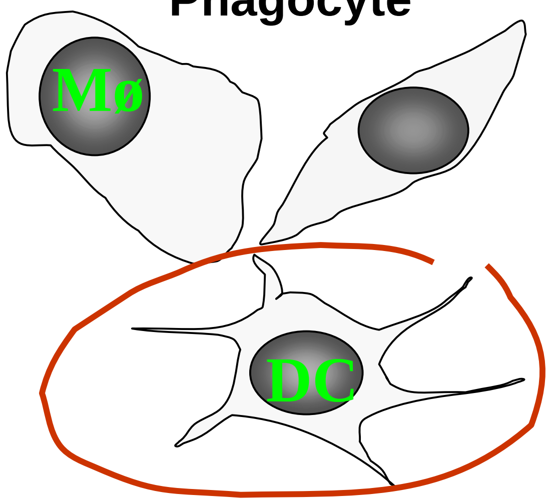

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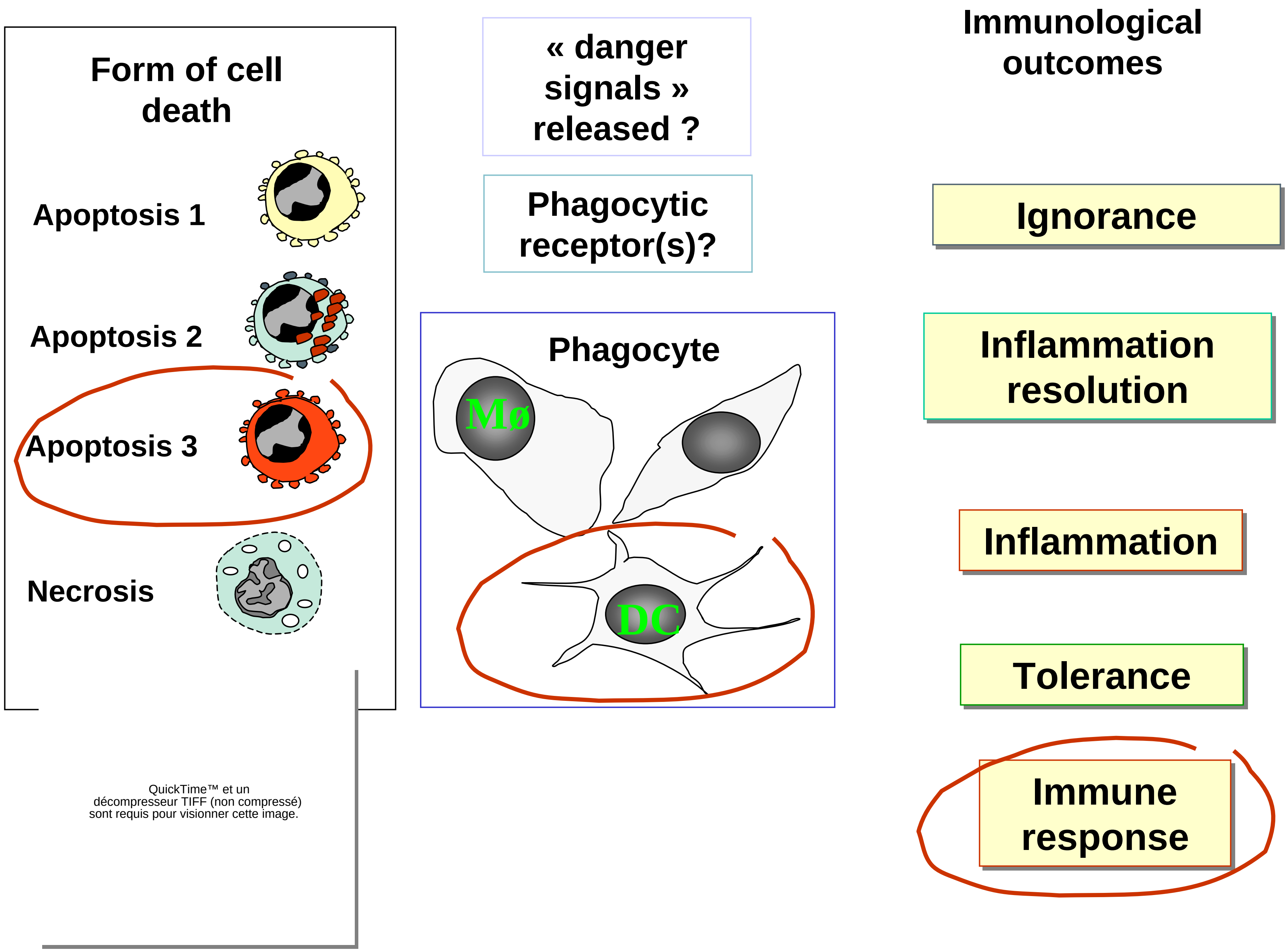
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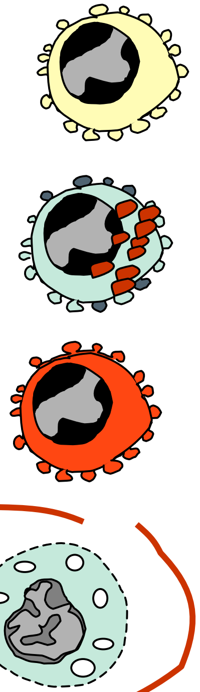

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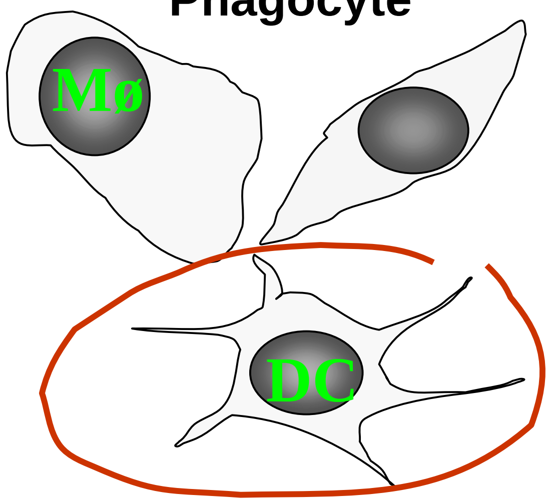

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Consequences of a defect in apoptotic cells phagocytosis (receptors k/o)

PSR -/- : « necrotic-like » cells accumulation in lungs but lethal (Science, 2003)

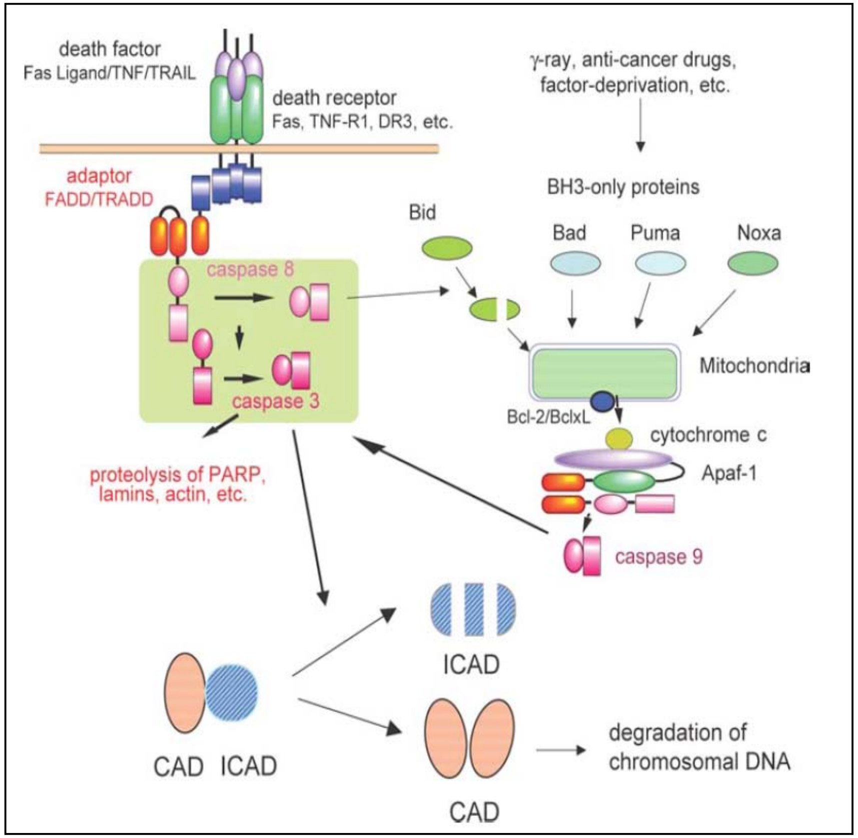
Mer -/- : delayed clearance of infused apoptotic cells and accumulation anti-ssDNA and anti-chromatin auto-antibodies (Cohen, J. Exp. Med, 2002)

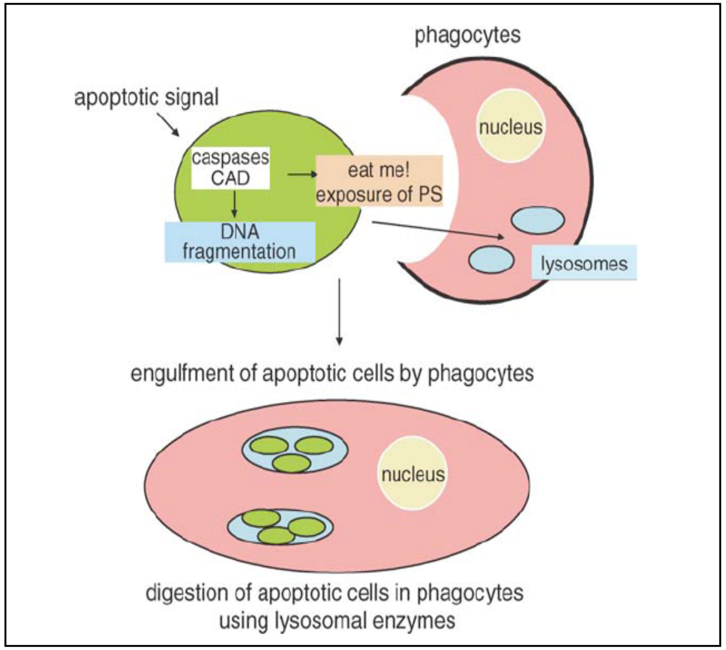
C1q -/- : accumulation apoptotic bodies in glomeruli and anti-nuclear antibodies (but not anti-ssDNA or anti-chromatin) and glomerulonephritis (kidney inflammation) (Botto, Nature Genetics 1998)

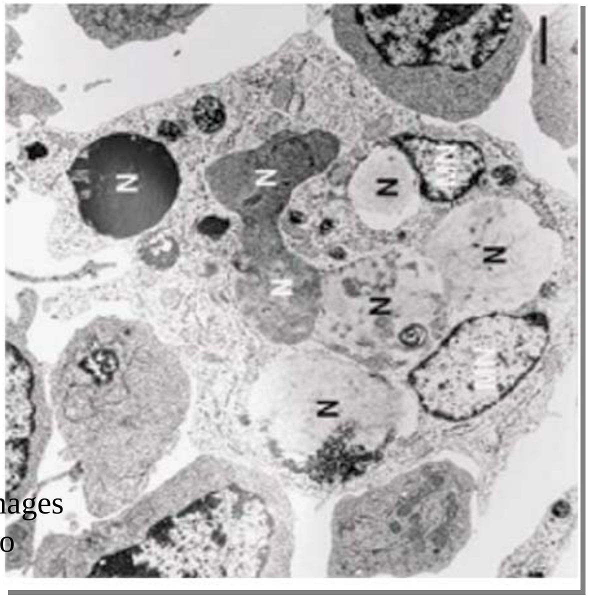
MFG-E8-/- :
- accumulation anti-nuclear antibodies and anti-dsDNA, glomerolonephritis (Hanayama, Science 2004)
- Impaired clearance of apoptotic mammary epithelial cells, Impaired involution mammary gland (Hanayama, PNAS 2005)

DNA degradation in Programmed Cell death

Shigekazu Nagata, Annu. Rev. Immunol. 2005. 23:853–75

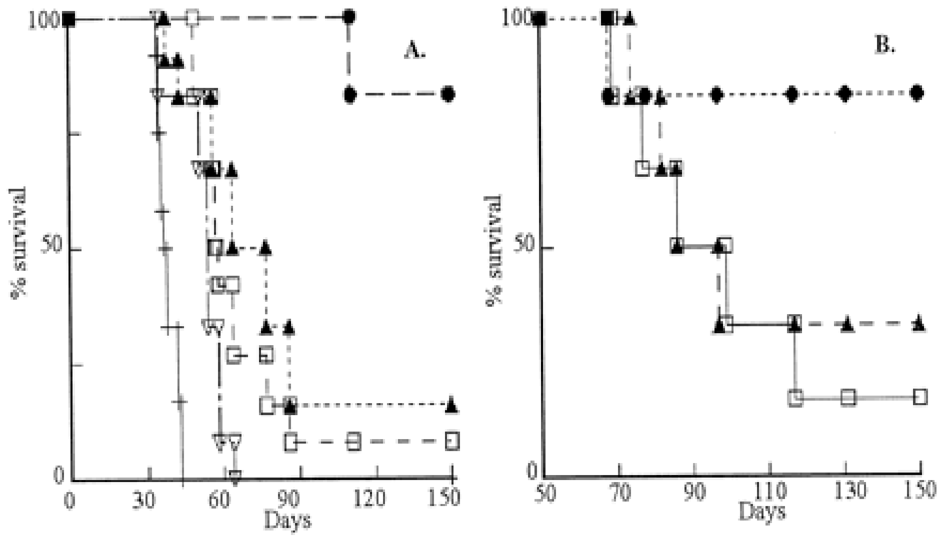






Accumulation of undigested nuclei in macrophages
from double knockout ICAD-DNase II embryo

Vaccination with phagocytic cells loaded with dead cells



Rats bearing adenocarcinomas injected i.p. 3 times with:

● peritoneal (A) or blood derived cells (B) + apoptotic cells + IL-2

▲ idem w/o apoptotic cells

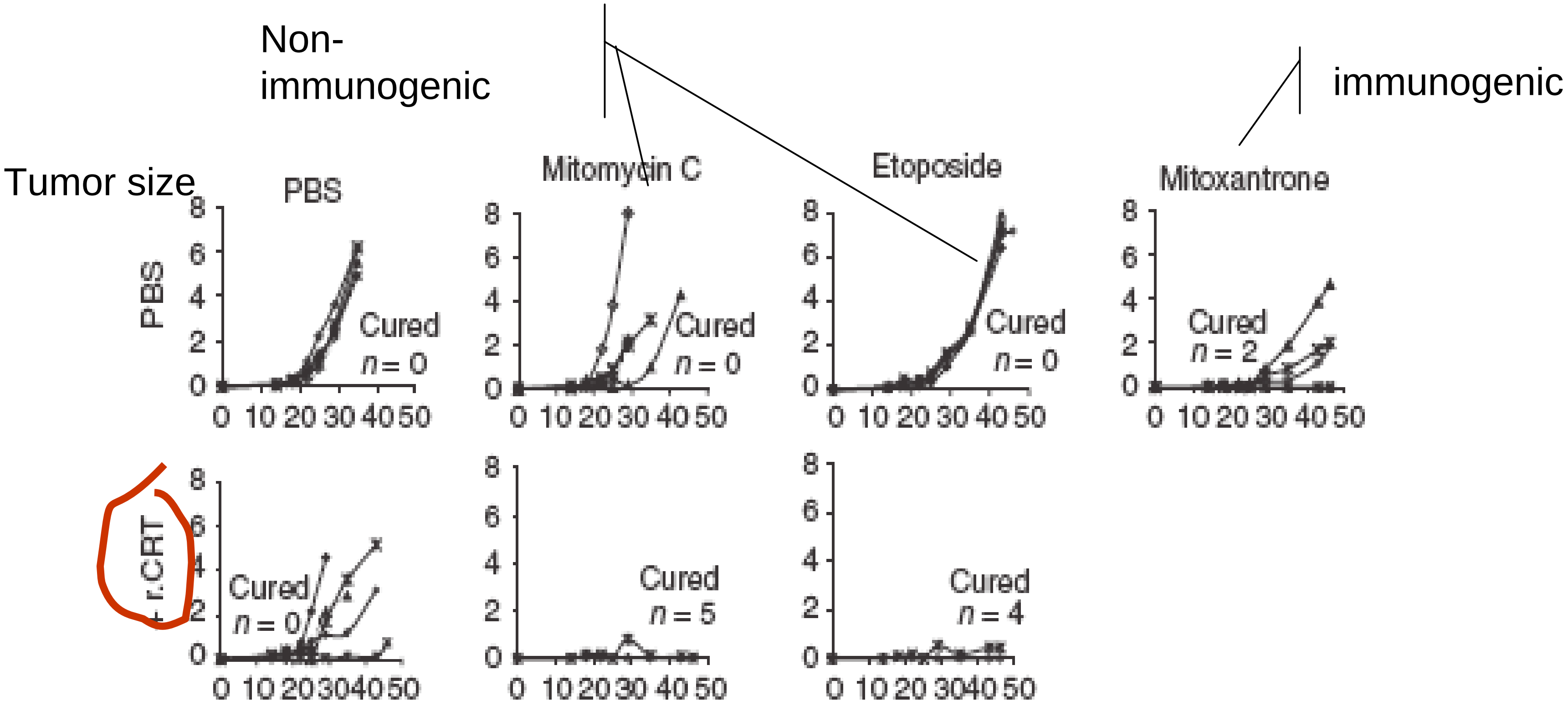
□ no treatment

Antigen-presenting Cells That Phagocytose Apoptotic Tumor-derived Cells Are Potent Tumor Vaccines

Frédéric Henry et al,, Cancer Research 1999

Therapeutic effect of chemotherapy with or without calreticulin

Mice with growing tumor: injection locally with chemotherapeutic agent with or without calreticulin

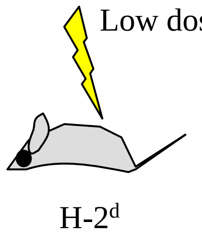


Protection only in immunocompetent mice **=> immune response**

CD4 and CD8 T cells necessary

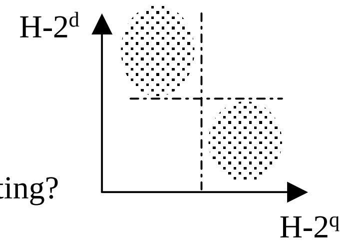
Bone marrow transplantation is facilitated by apoptotic cells injections

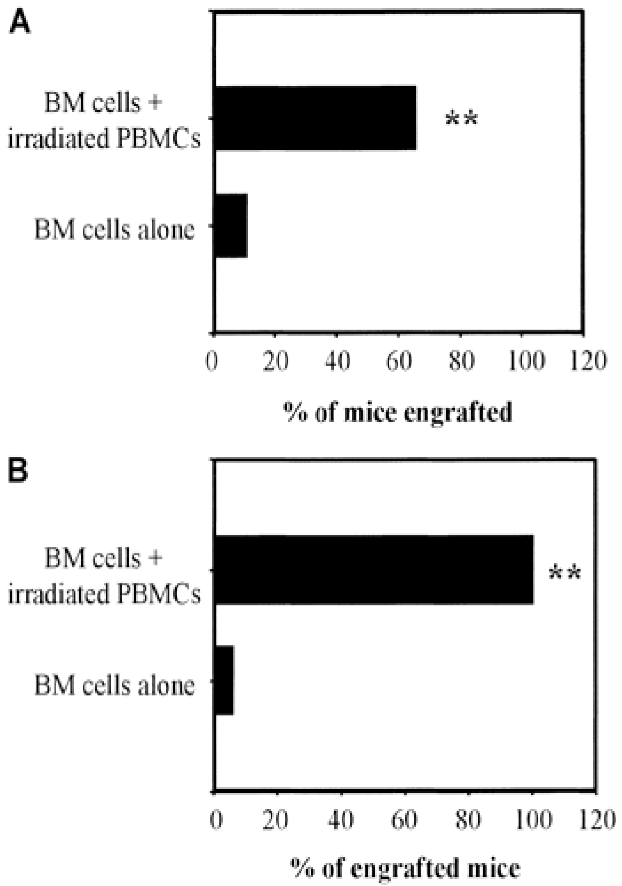
Host versus graft reactivity



Low number (10^6) BM cells H-2^q i.v. ====> Rejection

\+ apoptotic 5.10^6 spleen cells ====> Graft-facilitating?





Graft-facilitating effect of irradiated human PBMCs.

BALB/c (H-2d) mice were submitted to 6-Gy TBI and grafted 24 hours later with 10^6 BM cells from FVB (H-2qpanel A, 2 independent experiments with 12 mice per group) or C57BL/6 (H-2b; panel B, one experiment with 12 mice per group) mice alone or with 10^7-irradiated human PBMCs. Between day 45 and day 50 post-BMT, engraftment was measured by FACS analysis.

M. de Carvalho, Blood 2001; 98: 224-230