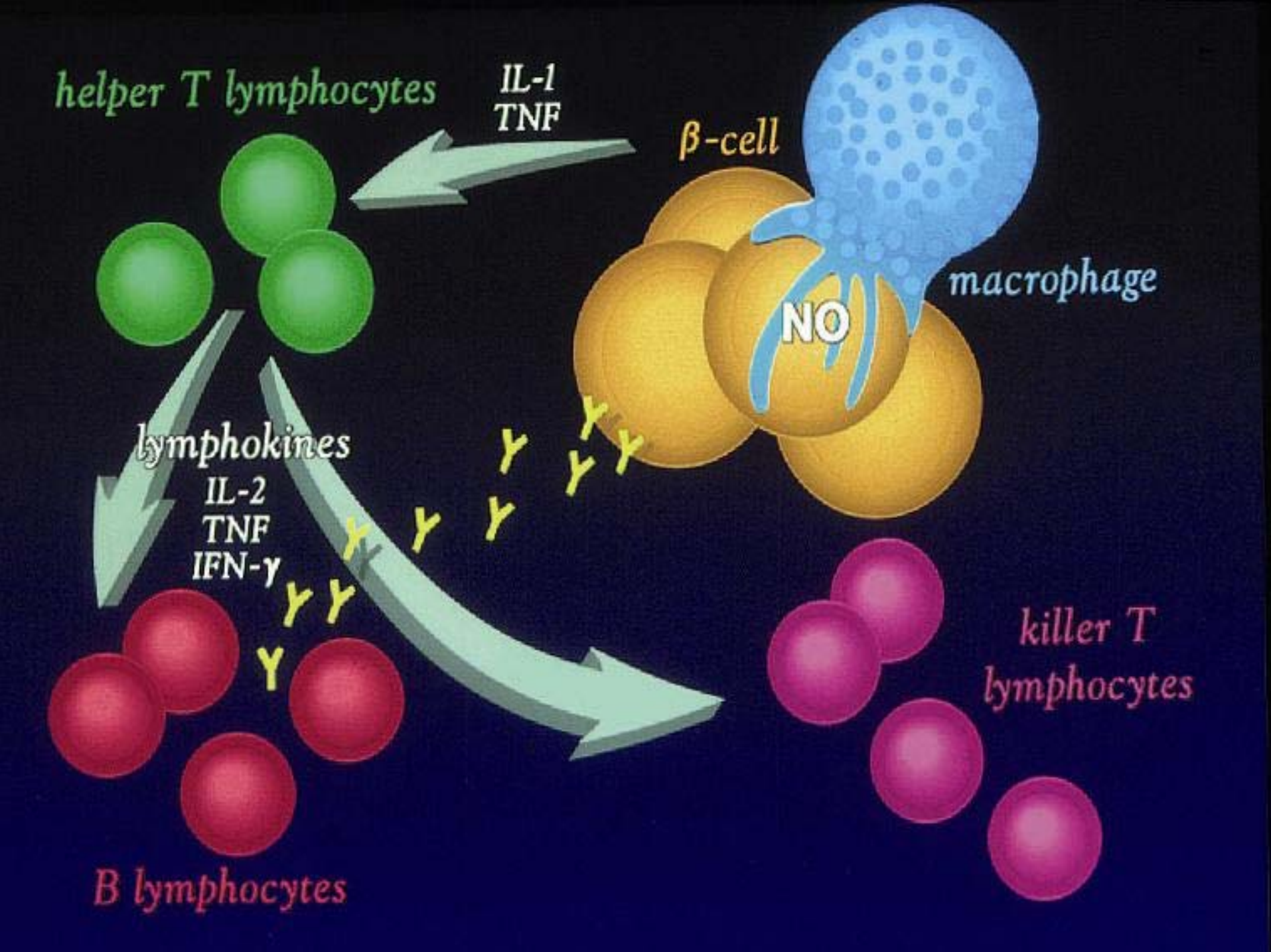
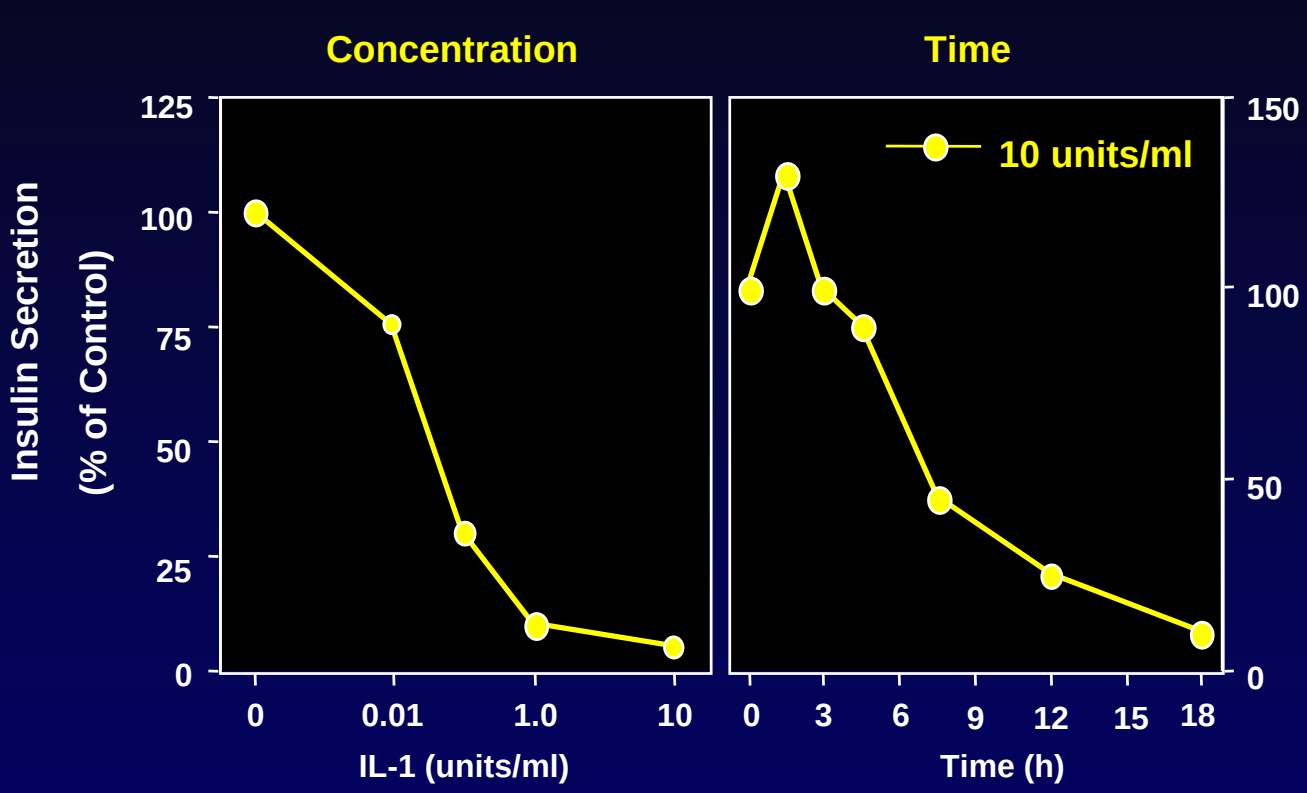
How do Cytokines Kill β-cells?

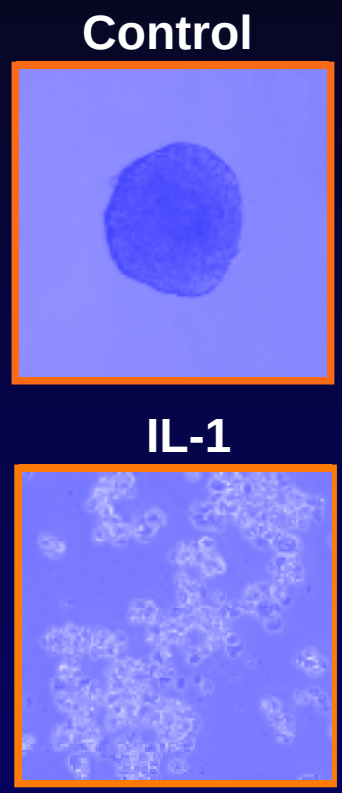
Necrosis, Apoptosis, and Nitric Oxide

John A Corbett, Saint Louis University

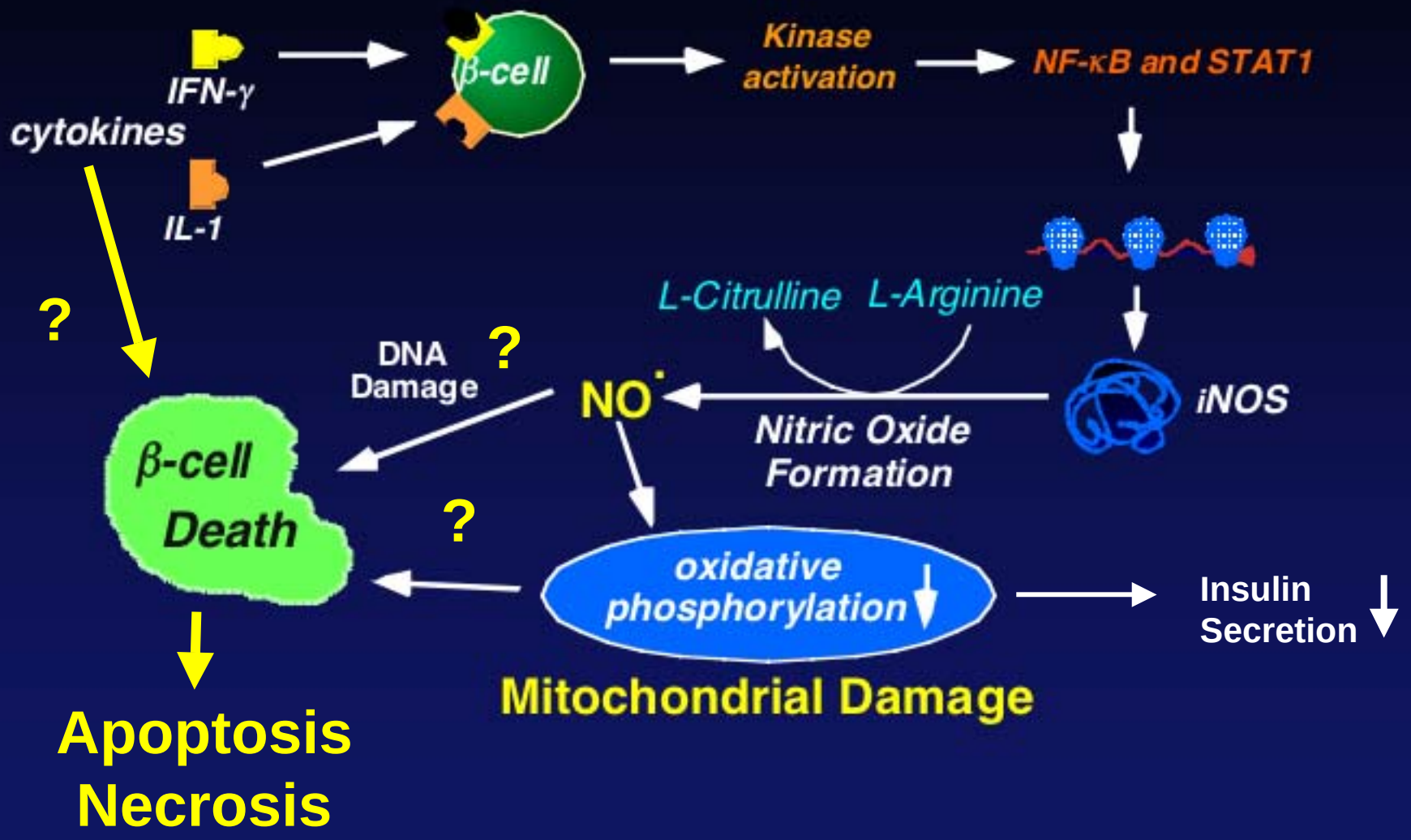

helper T lymphocytes
IL-1
TNF
β-cell
macrophage
NO
lymphokines
IL-2
TNF
IFN-γ
killer T
lymphocytes
B lymphocytes

IL-1 Inhibits Glucose-induced Insulin Secretion by Rat islets





Cytokine-mediated β-cell Damage: Rodent & Human islets



Mechanisms of cytokine-mediated β-cell death

Literature Survey: 1996-2006

Key words	Citations
Islet, Cytokine, Apoptosis	145
Islet, Cytokine, Apoptosis, Nitric Oxide	68
Islet, Cytokine, Necrosis	185
Islet, Cytokine, Necrosis, Nitric Oxide	68

Results: Cytokine-induced β-cell death is by nitric oxide-dependent and-independent apoptotic and necrotic pathways.

Many recent papers suggest that cytokines kill by nitric oxide-independent apoptosis.

Necrosis

Form of cell death that stimulates inflammation

Characterized by cell swelling and lysing

Mitochondrial swelling, loss of plasma membrane integrity

Cell content is released in an uncontrolled manner

Apoptosis

Form of cell death that does not stimulate inflammation.

Energy-driven process-cell actively destroys itself in response to extracellular signals or developmental cues

ordered degradation of proteins and organelles, chromatin condensation, nuclear fragmentation

maintenance of plasma membrane integrity

Effects of IL-1 on β-cell Viability

MTT Assay: metabolic activity

Cleavage of the tetrazolium salt MTT to yield formazan by metabolically active cells

Neutral Red Assay: membrane integrity

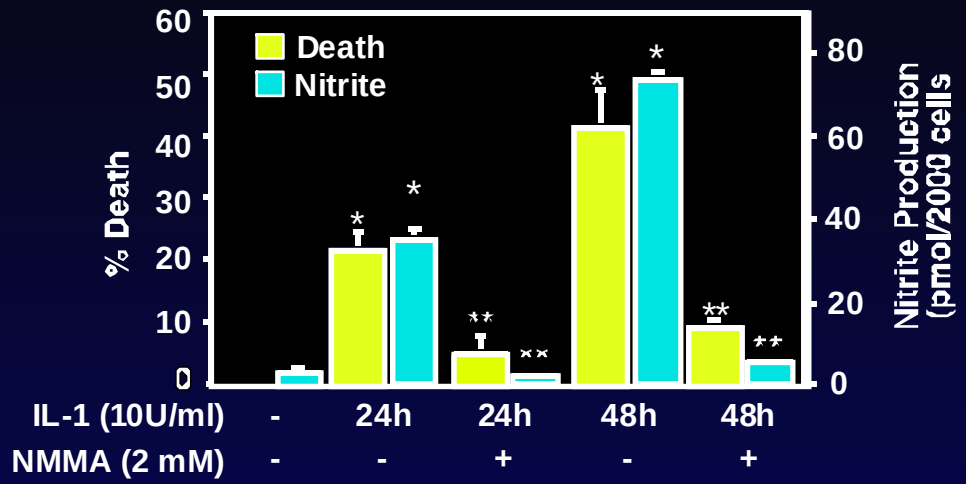
Neutral Red is a weak cationic dye that readily penetrates cell membranes by non-ionic diffusion, accumulating in lysosomes.

TUNEL Assay: DNA integrity

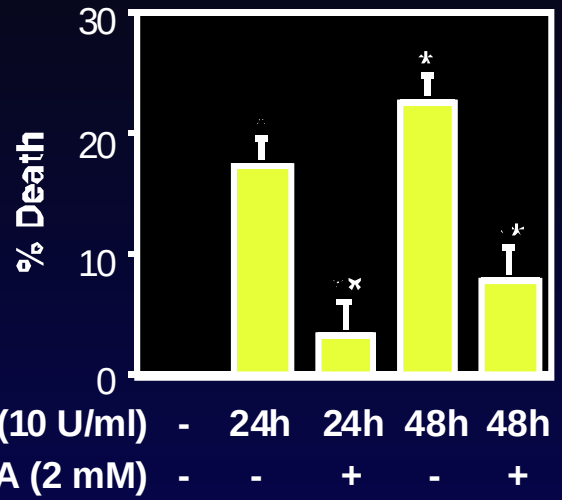
(terminal dUTP nick-end labelling) double-stranded DNA fragments as well as single strand breaks (“nicks”) detected by enzymatic labeling of the free 3’-OH termini with modified nucleotides (X-dUTP, X = biotin, DIG or fluorescein).

Effects of IL-1 on β-cell cell Viability:MTT/NR

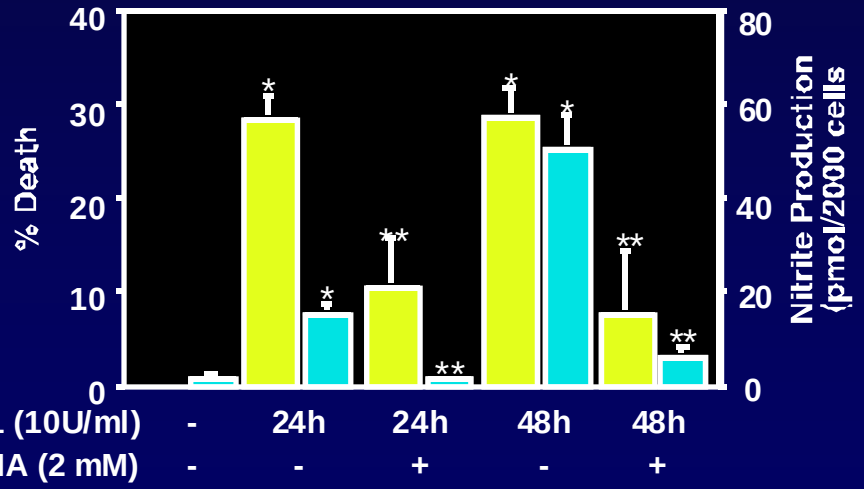
A) RINm5F Cells



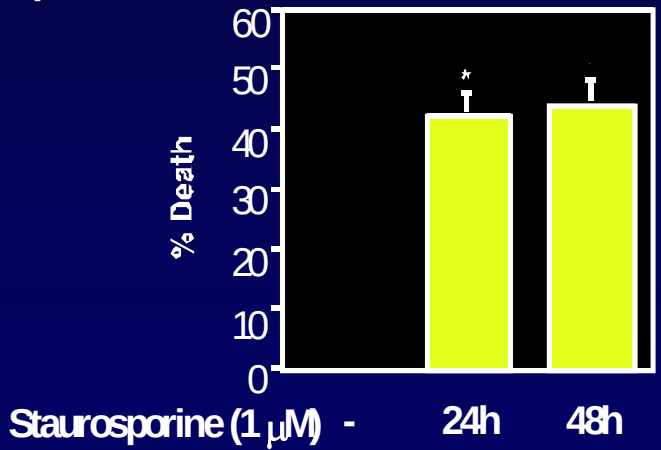
B) RinM5F Cells: Neutral Red Uptake



C) Rat Islets

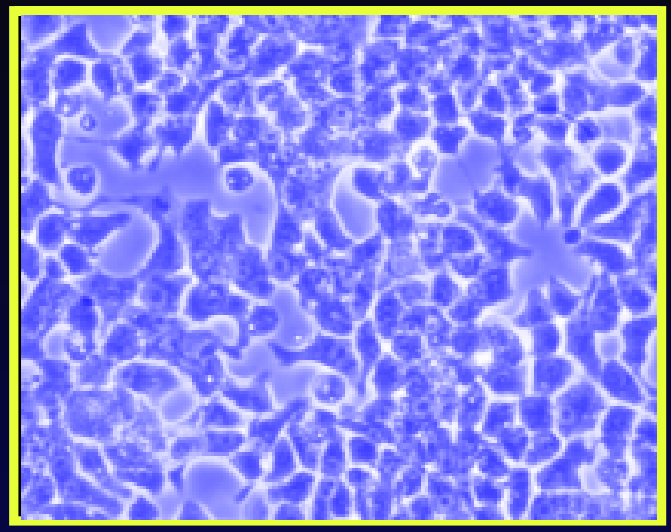


D) Rat Islets

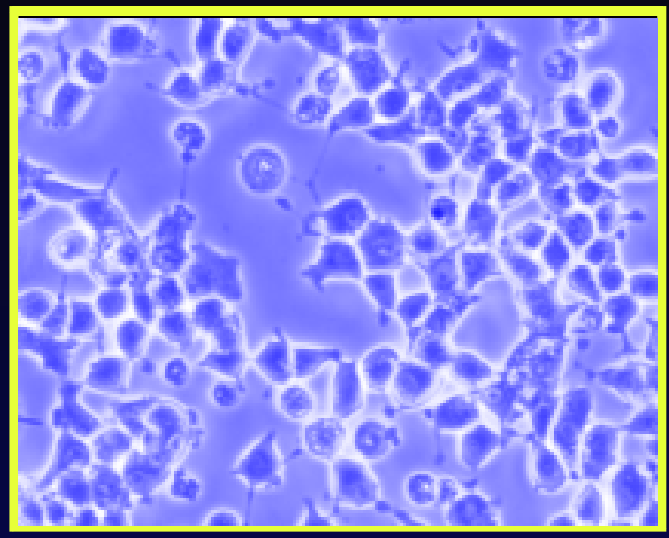


Effects of IL-1 on RINm5F cell Morphology

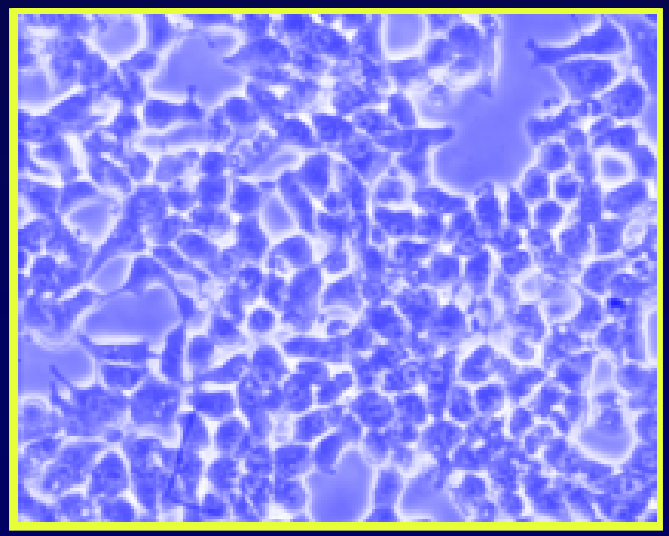
A) Control



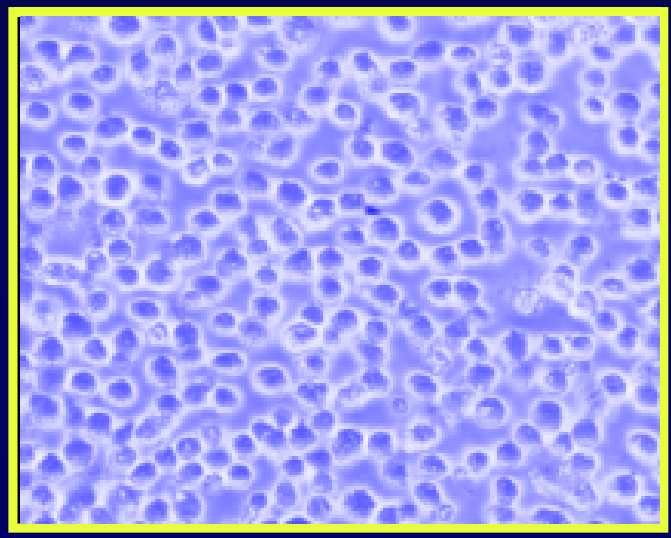
B) IL-1



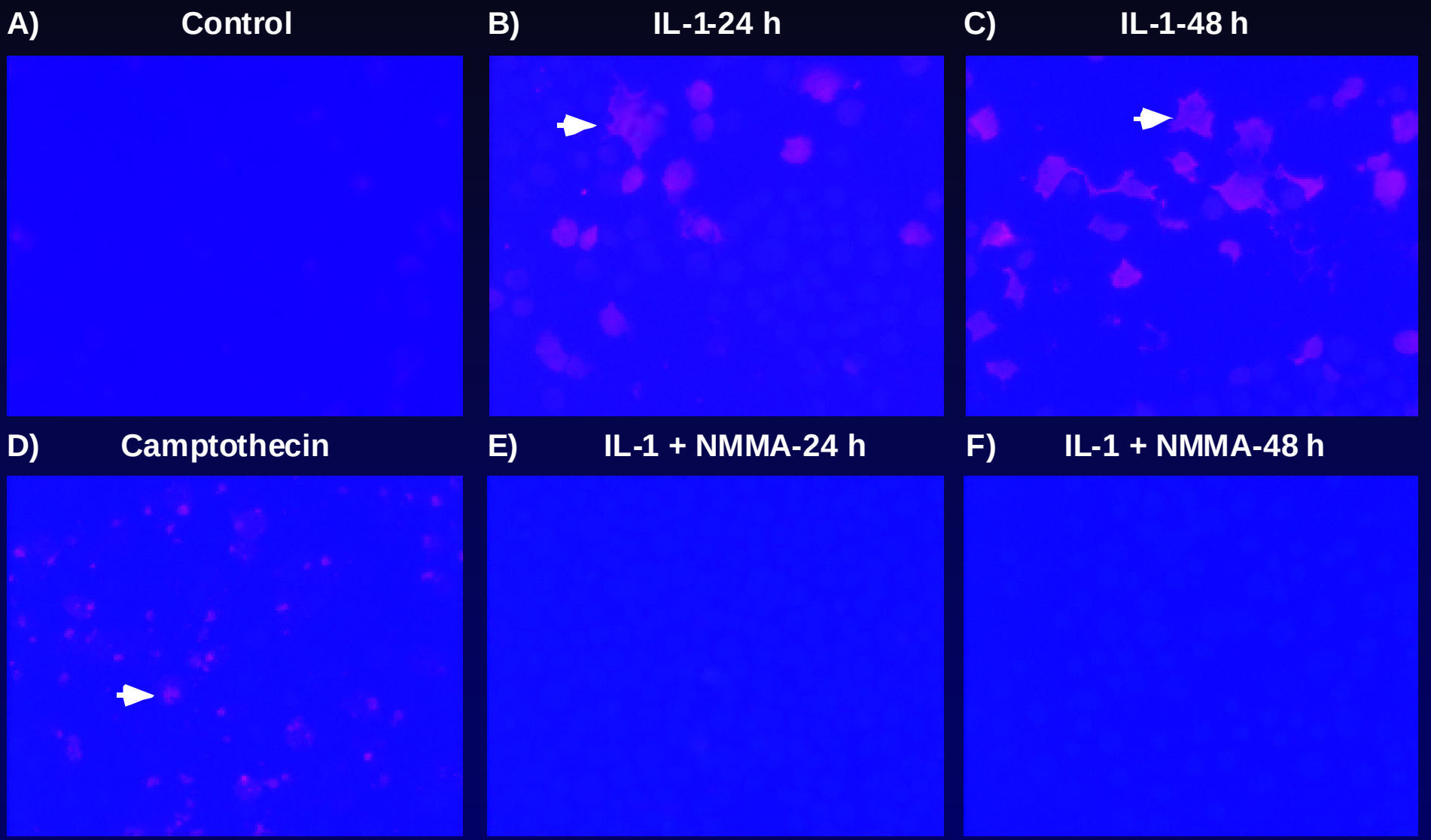
C) IL-1 + NMMA



D) Camptothecin



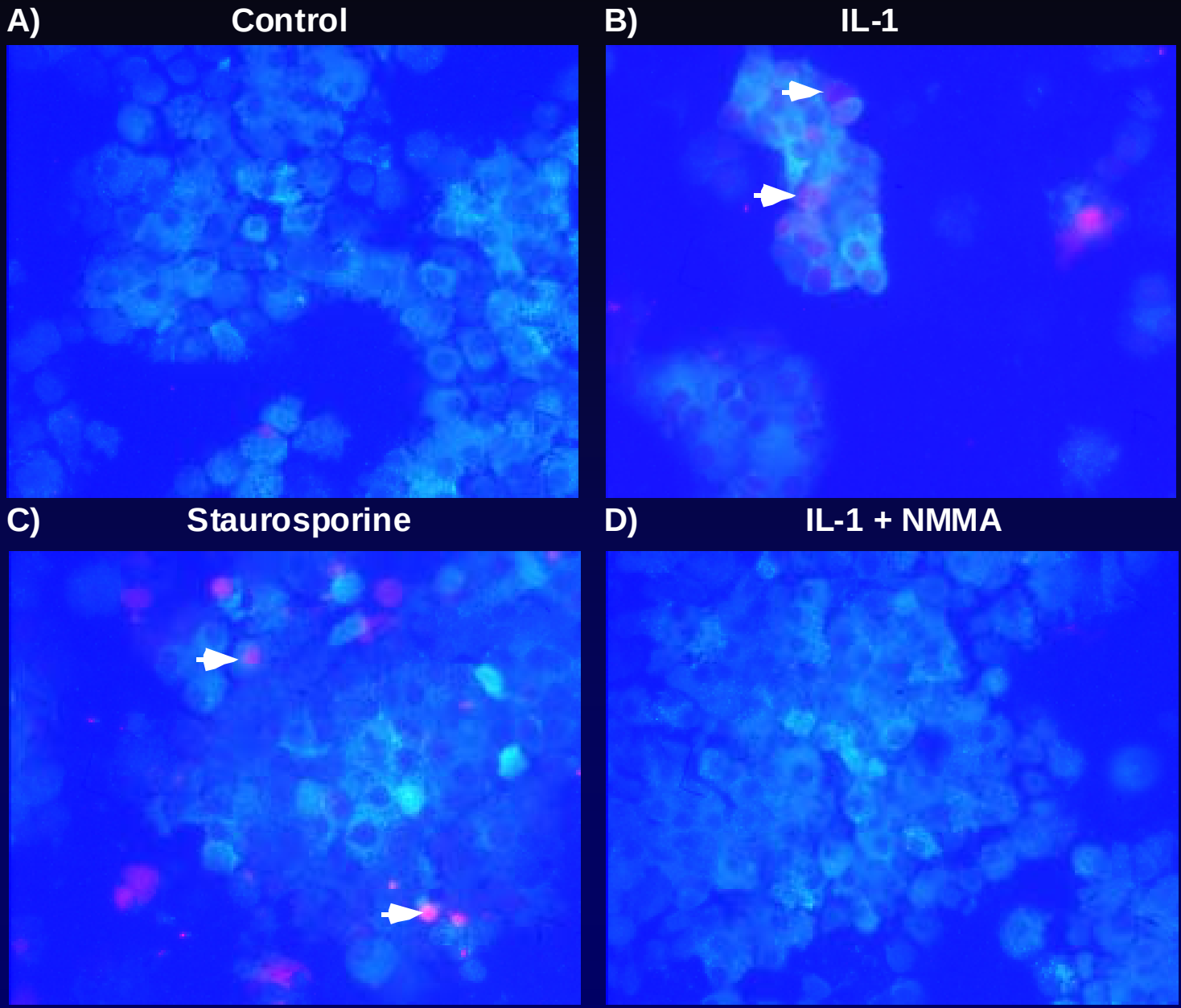
Effects of IL-1 on RINm5F cell DNA Integrity: TUNEL Staining



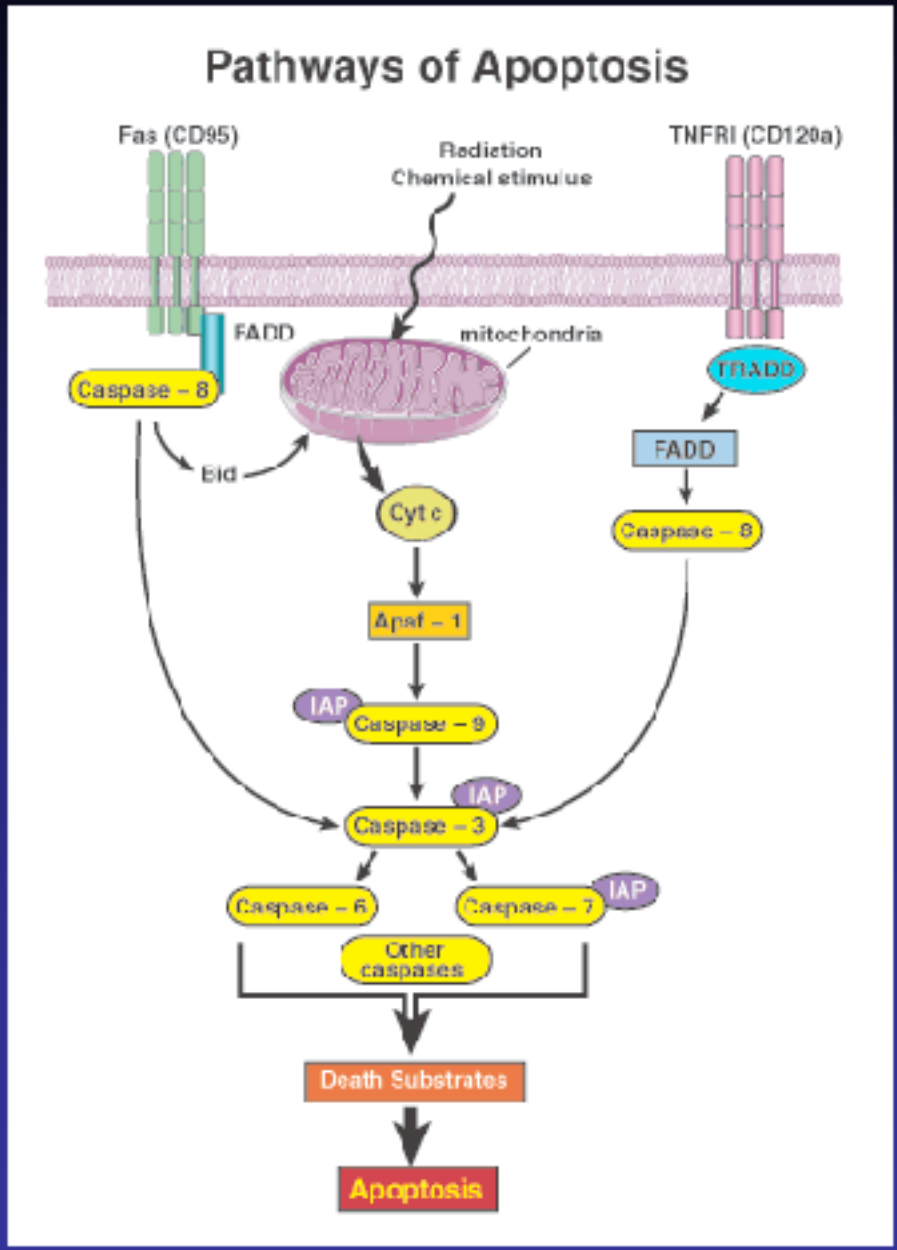
Effects of IL-1 on β-cell cell DNA Integrity: TUNEL Staining

Red:Insulin

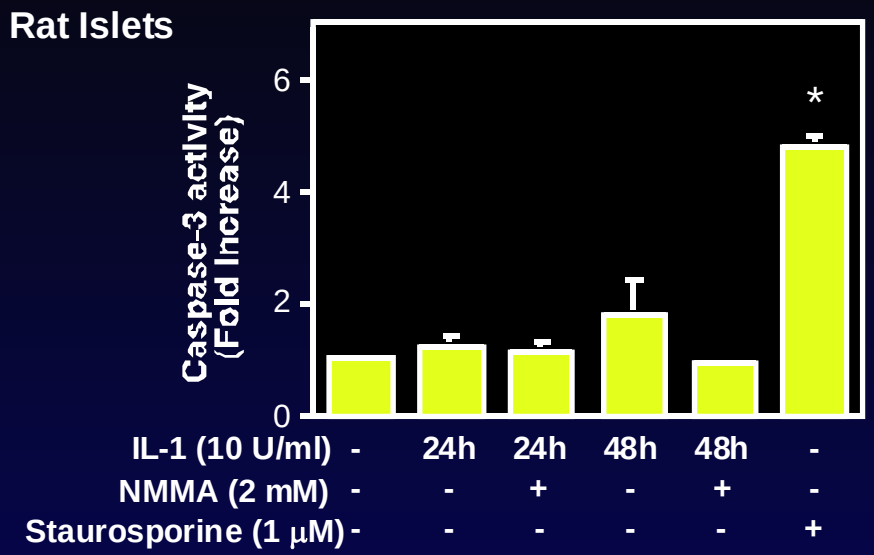
Green: TUNEL

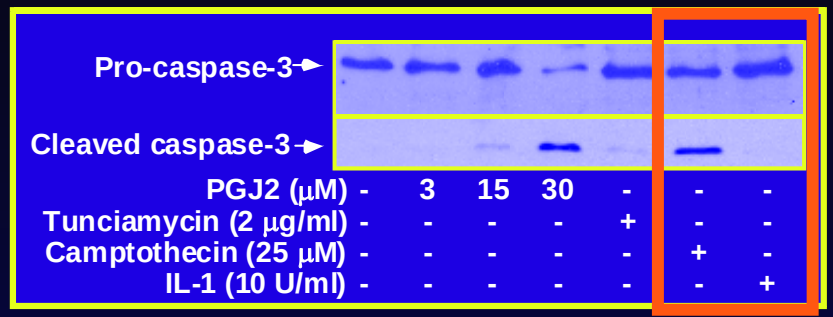


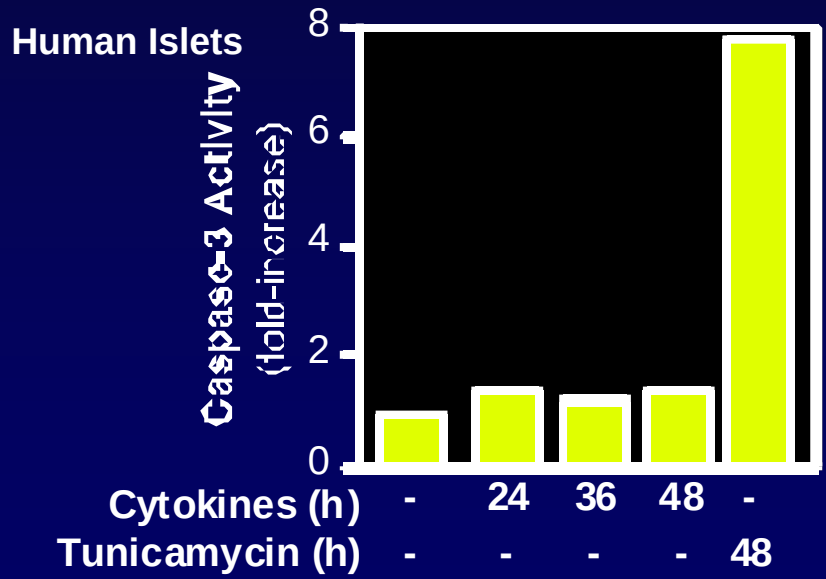
Does IL-1 induce "classical" β-cell apoptosis?



IL-1 Fails to activate Caspase 3

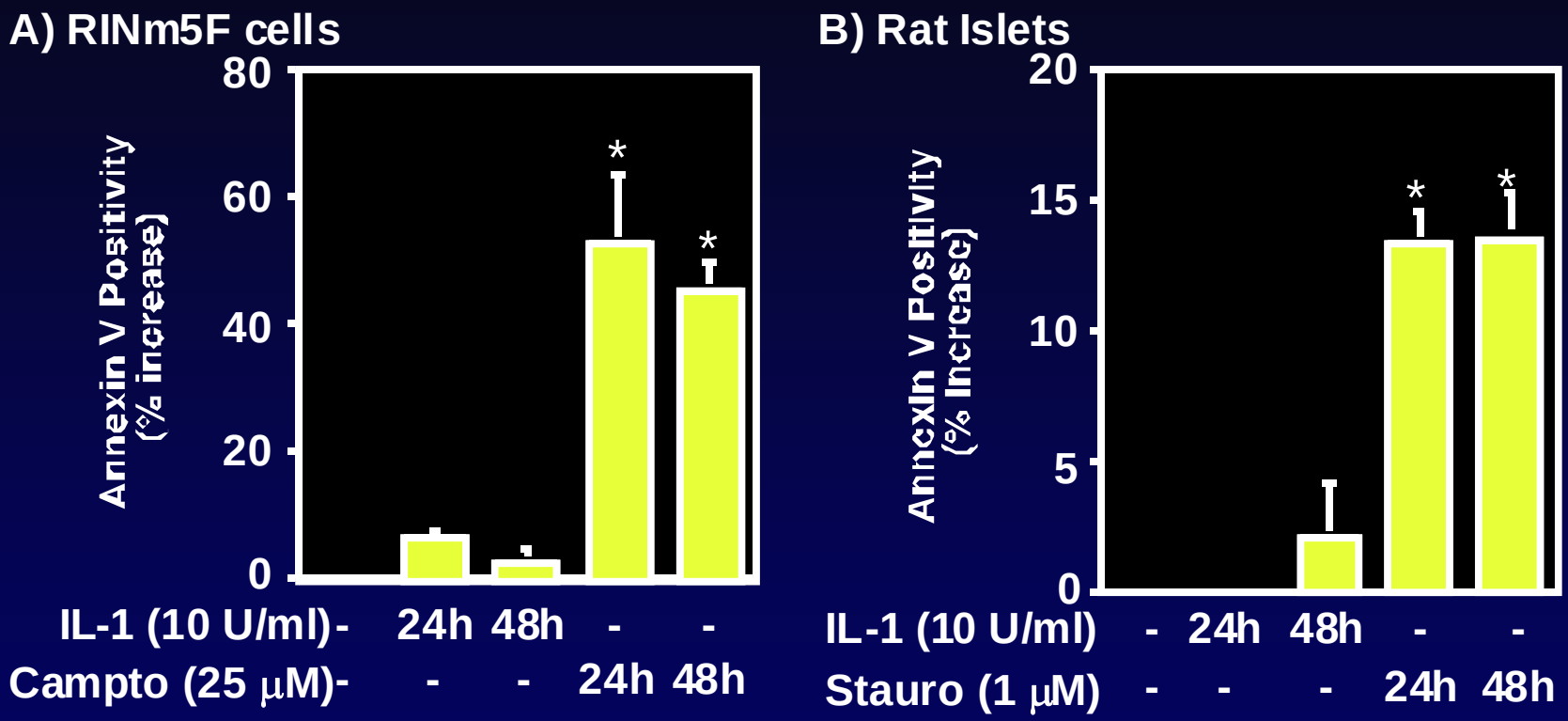




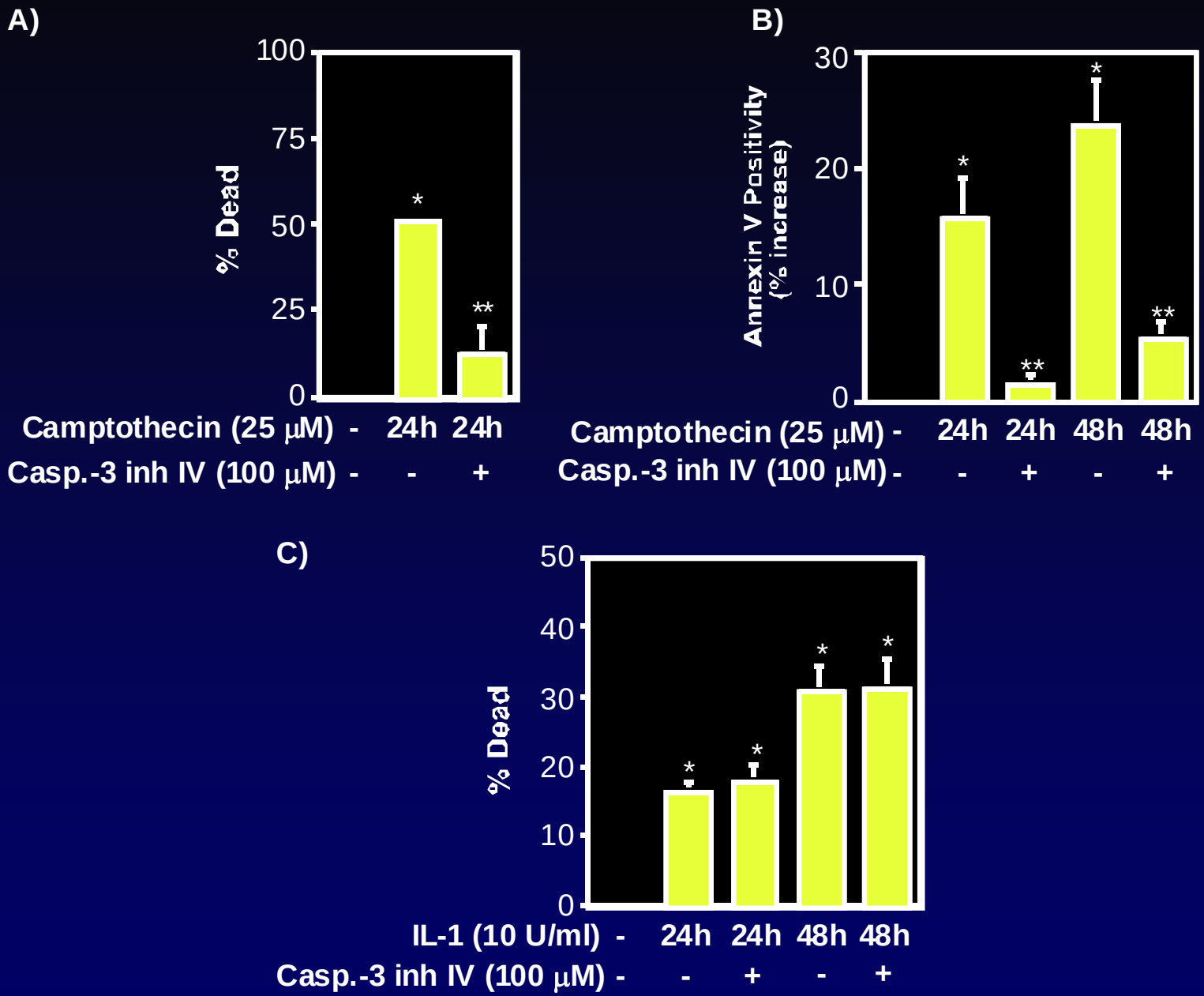


IL-1 75 units/ml
IFN-γ 500 units/ml
TNF 10 (nM)

IL-1 fails to stimulate phosphatidyl serine externalization



Effects of Caspase 3 inhibition on β-cell viability



Nitric Oxide and "Classical" Apoptosis

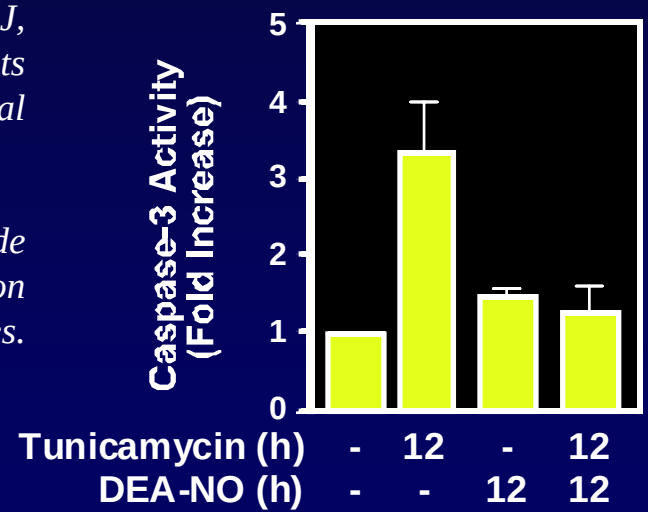
1. Apoptosis requires ATP while nitric oxide reduces islet ATP levels 4-fold

Corbett, JA, Wang, JL, Hughes, JH, Wolf, BA, Sweetland, MA, Lancaster, JR, Jr., and McDaniel, ML. 1992. Nitric Oxide and cGMP Formation Induced by Interleukin 1β inIslets of Langerhans: Evidence for an Effector Role of Nitric Oxide in β-cell Dysfunction. Biochem. J. 287, 229-235

2. Nitric oxide is an inhibitor of caspase activity

Rossig L, Fichtlscherer B, Breitschopf K, Haendeler J, Zeiher AM, Mulsch A, Dimmeler S. Nitric oxide inhibits caspase-3 by S-nitrosation in vivo. Journal of Biological Chemistry 1999;274(11):6823-6.

Li J, Bombeck CA, Yang S, Kim YM, Billiar TR. Nitric oxide suppresses apoptosis via interrupting caspase activation and mitochondrial dysfunction in cultured hepatocytes. Journal of Biological Chemistry 1999;274(24):17325-33.



HMGB1 A Marker for “Programmed Necrosis”

Zong et al., Gene & Dev. 18:1272, 04

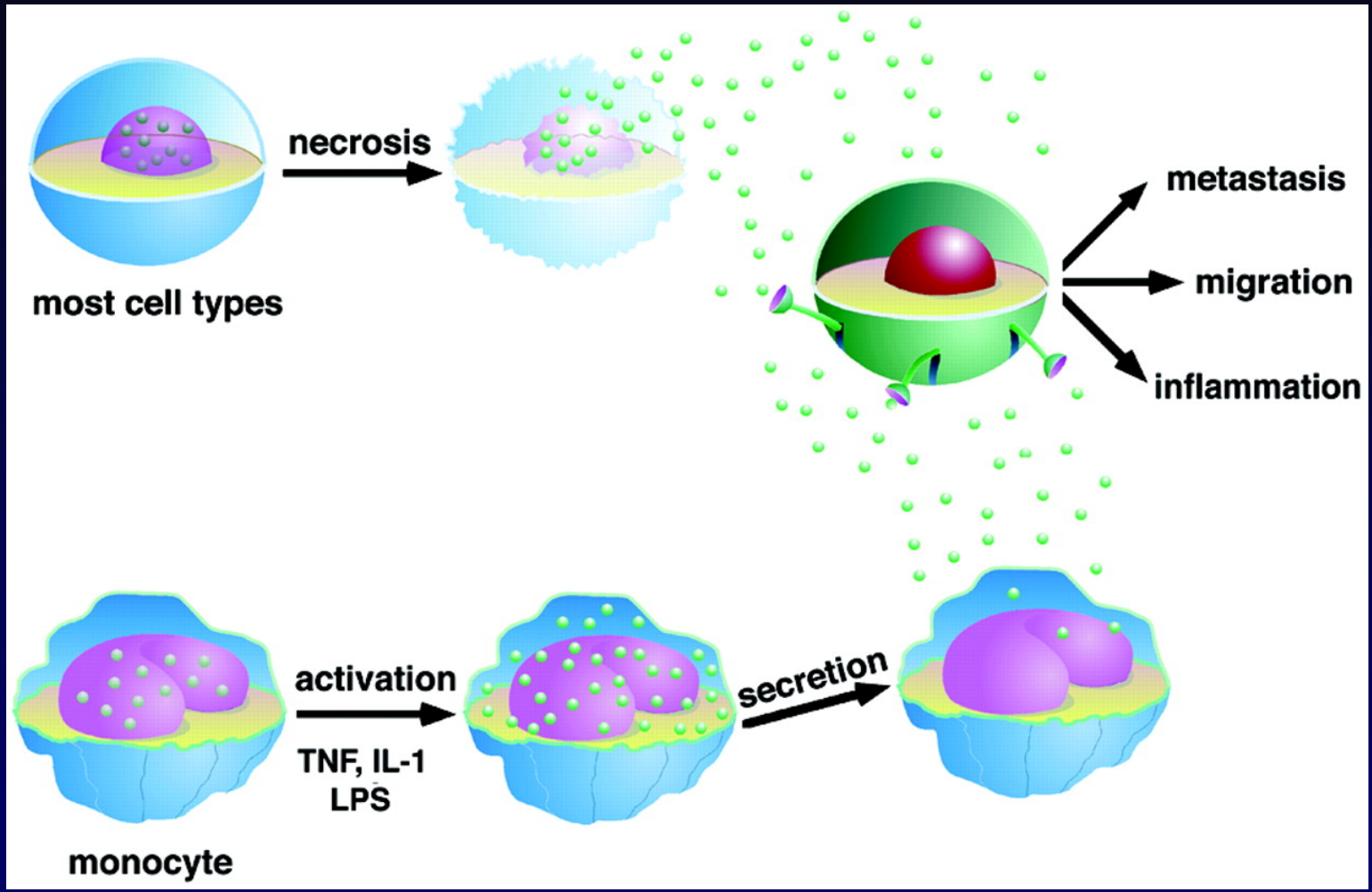
1. Small acidic chromatin binding protein.

2. HMGB1 proinflammatory mediator enhances inflammation induced by necrotic cells (Scaffidi et al., Nature 418: 191, 02)

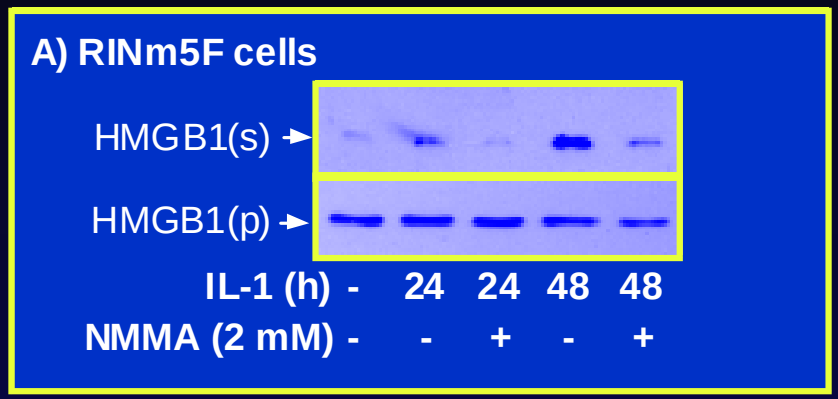
3. Under conditions of DNA damage- HMGB1 redistributed from nucleus to cytosol (acetylation/ribosylation).

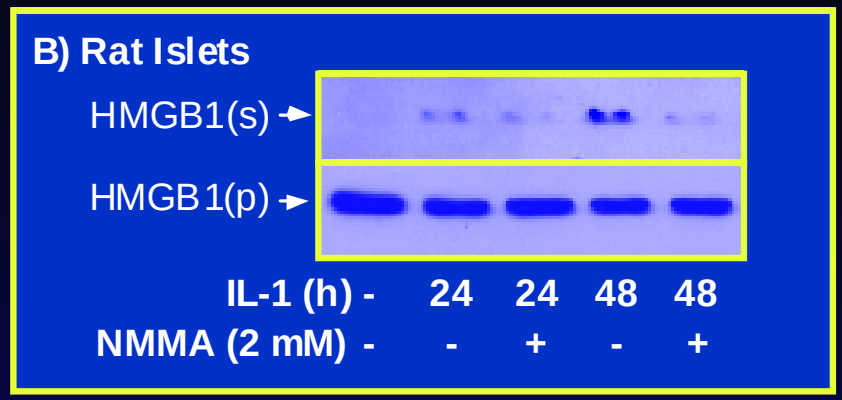
4. During apoptosis: it has an affinity for apoptotic DNA fragments and is not released (Muller et al., EMBO J. 20:4337, 01)

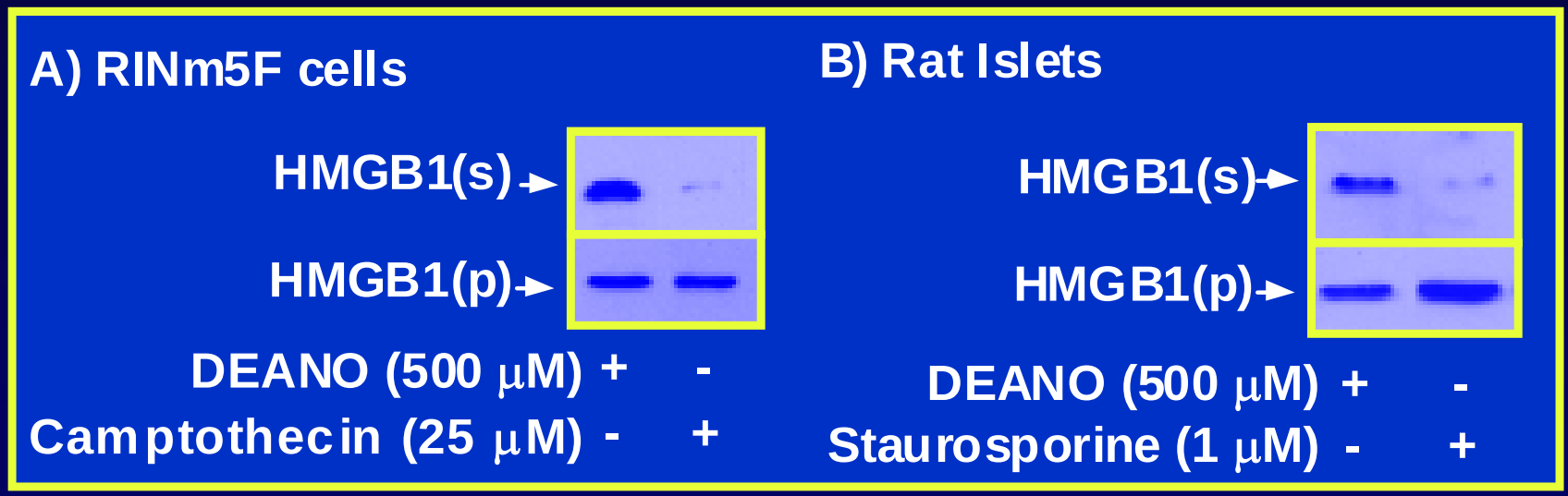
Multiple immunological roles of HMGB1



IL-1 stimulates the nitric oxide-dependent release of HMGB1 from β-cells







Do Caspase Inhibitors prevent β-cell necrosis?

Effects of caspase 3 inhibitors on IL-1- and nitric oxide-induced β-cell death and HMGB1 release

Caspase 3 inhibition fails to prevent Nitric oxide or IL-1-induced HMGB1 release from β-cells

A) RINm5F

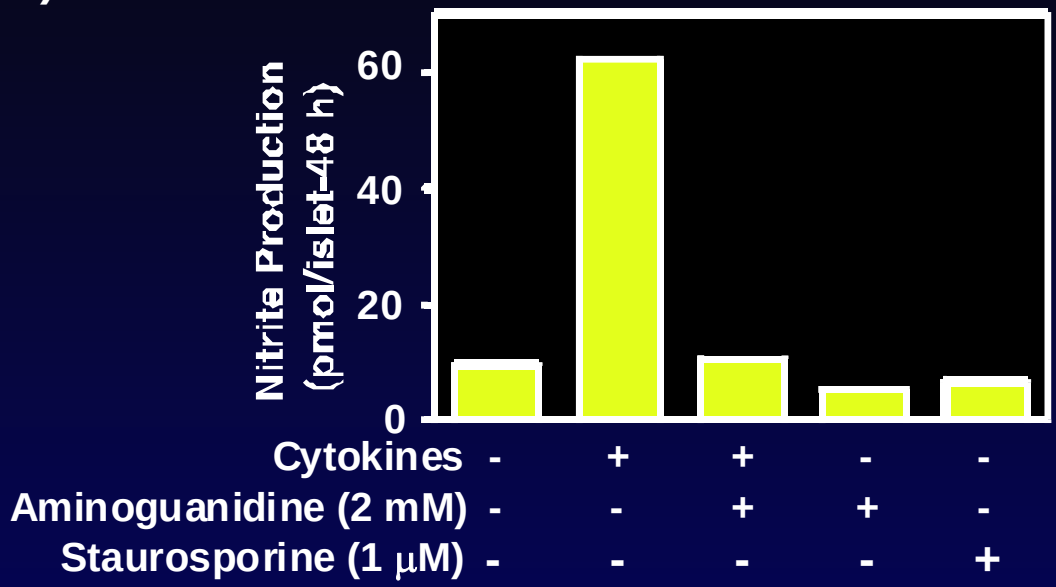
DEANO (500 μM)	-	+	+
Casp.-3 inh IV (100 μM)	-	-	+

B) RINm5F

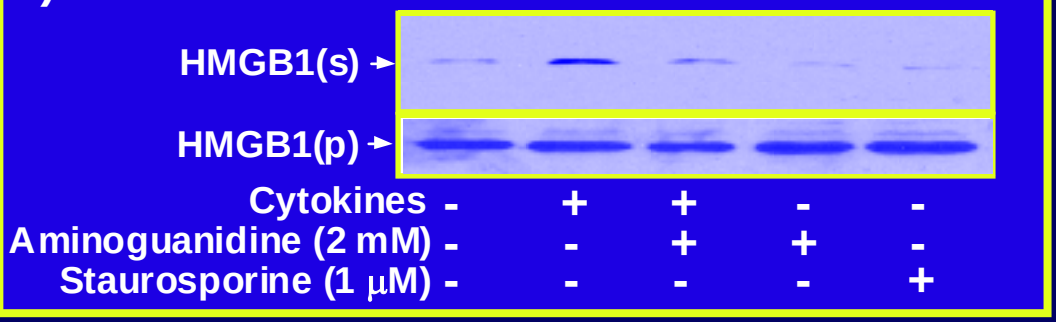
IL-1 (10 U/ml)	-	+	+
Casp.-3 inh IV (100 μM)	-	-	+

Cytokine combination of IL-1, TNF, and IFN-γ stimulate HMGB1 Release from Human Islets

A) Nitric Oxide Production



B) HMGB1 Release



*Results observed in 3 of 5 independent human islet preparation.

Summary of cytokines and β-cell death

Evidence in favor of cytokine-induced β-cell apoptosis:

TUNEL Staining: DNA damage

Evidence in favor of cytokine-induced β-cell necrosis:

Morphological features consistent with necrosis

Inhibition of β-cell oxidative metabolism (ATP is required for apoptosis and nitric oxide reduces islet ATP content 4-fold).

Lack of caspase activation or cleavage or inhibition of IL-1-induced islet cell death by caspase inhibitors

IL-1 induces the release of HMGB1 a marker of necrosis

Final thoughts concerning HMGB1 and the Initiation of Autoimmune Diabetes

HMGB1 and antigen presentation:

HMGB1-deficient cells have a reduced capacity to activate APCs

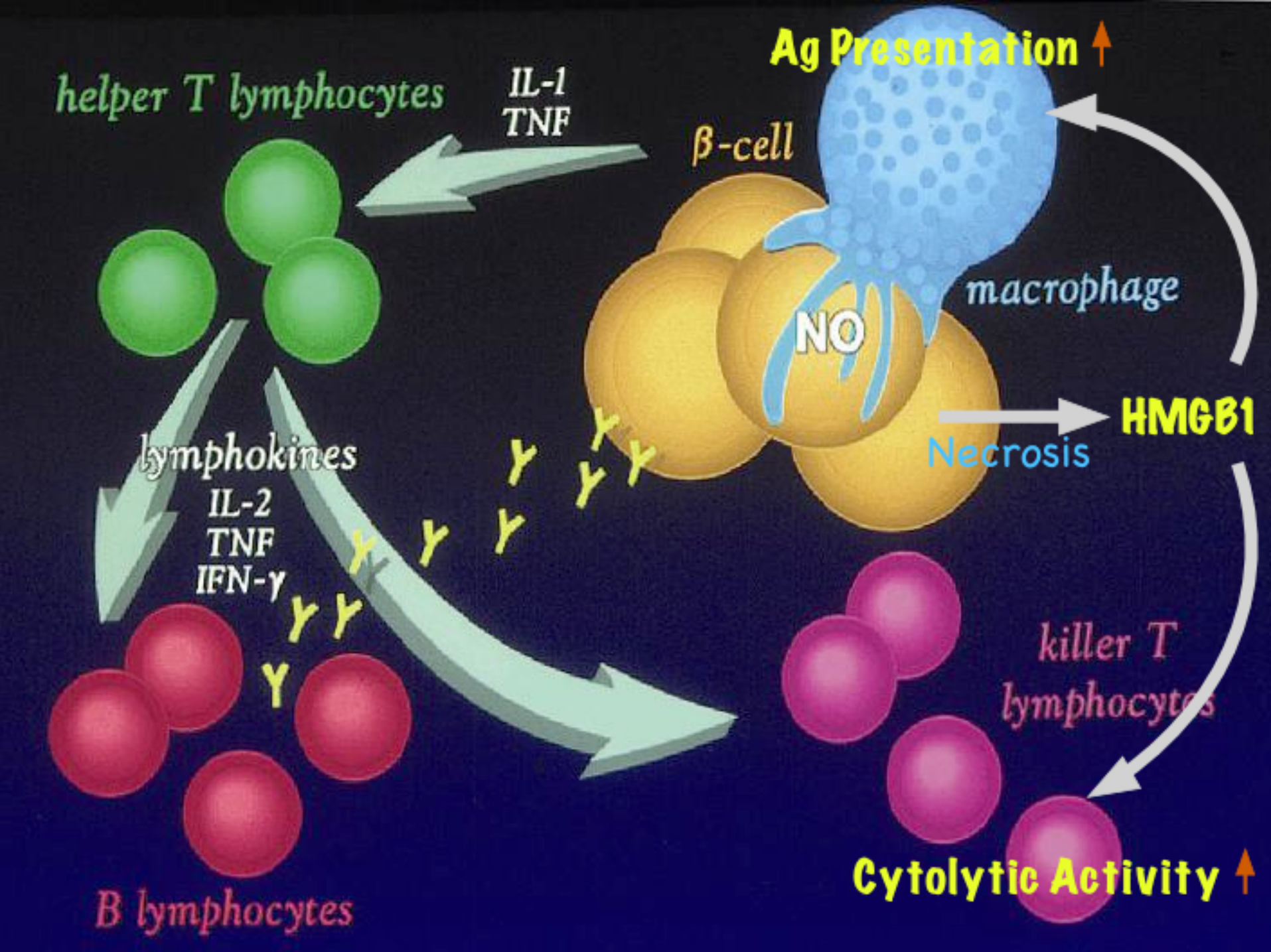
HMGB1 neutralization attenuates the ability of supernatants derived from necrotic cells to activate APC

HMGB1 and T-cells

HMGB1 enhances primary antibody responses to soluble antigens

HMGB1 enhances cytotoxic lymphocyte responses

Rovere-Querini P, Capobianco A, Scaffidi P, Valentinis B, Catalanotti F, et al. (2004) HMGB1 is an endogenous immune adjuvant released by necrotic cells. EMBO Rep 5: 825-830.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lymphocytes
B lymphocytes
Cytolytic Activity

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Colleen Kelly

Washington University

DRTC

Michael McDaniel