

# **Mechanisms of ART Liver Injury**

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# Drug-Induced Liver Injury (DILI)

- Leading cause of ALF in US
- Major reason drugs are not approved, restricted or withdrawn from market
  - CCR5 antagonists aplaviroc (Glaxo) and maraviroc (Pfizer)
- DILI from ART may result in morbidity/mortality and also limit/interrupt effective therapy
- Mechanisms of DILI are poorly understood
  - No small animal models
  - Relatively rare
  - No diagnostic tests

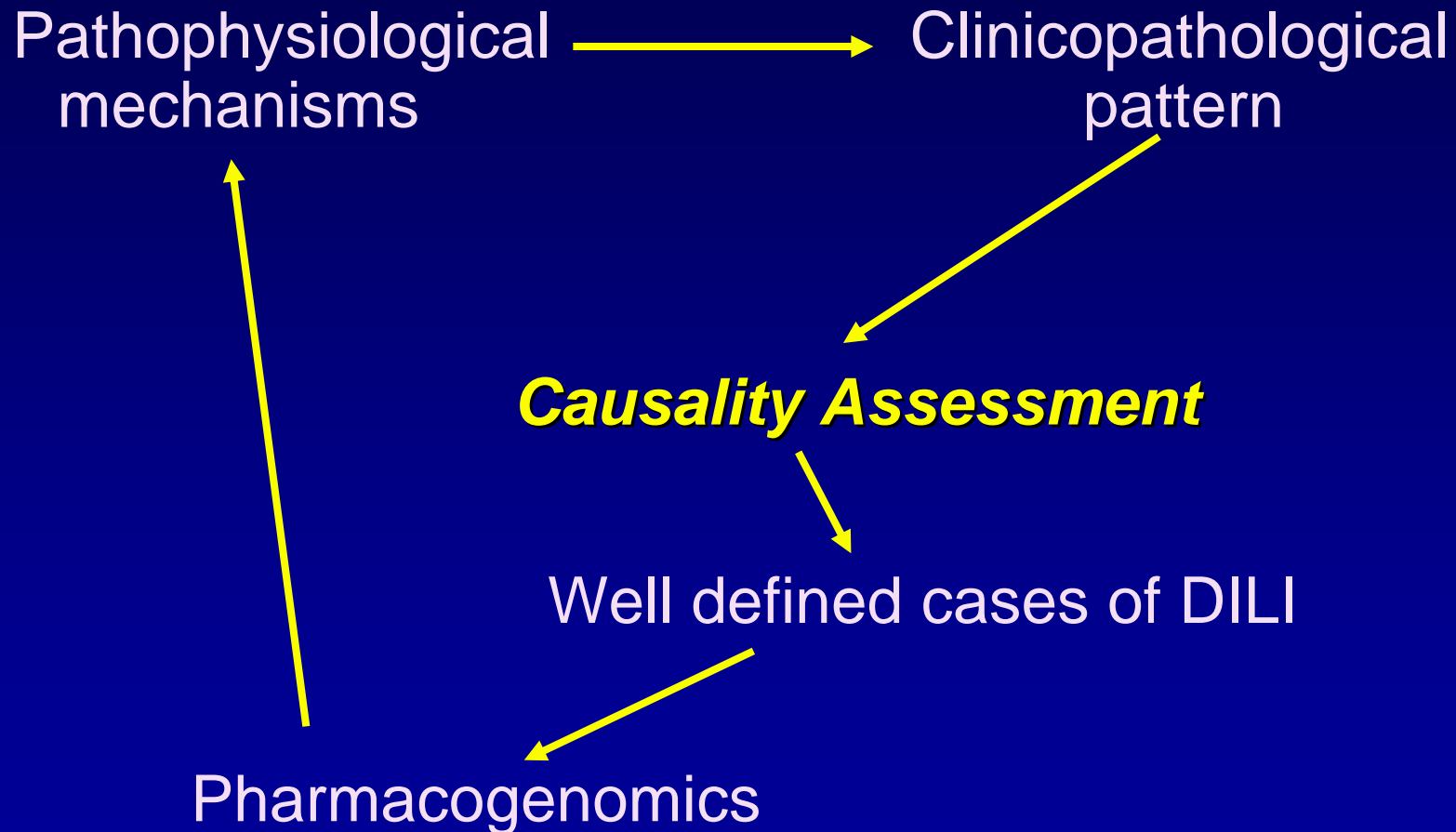
# Mechanisms of ART Liver Injury

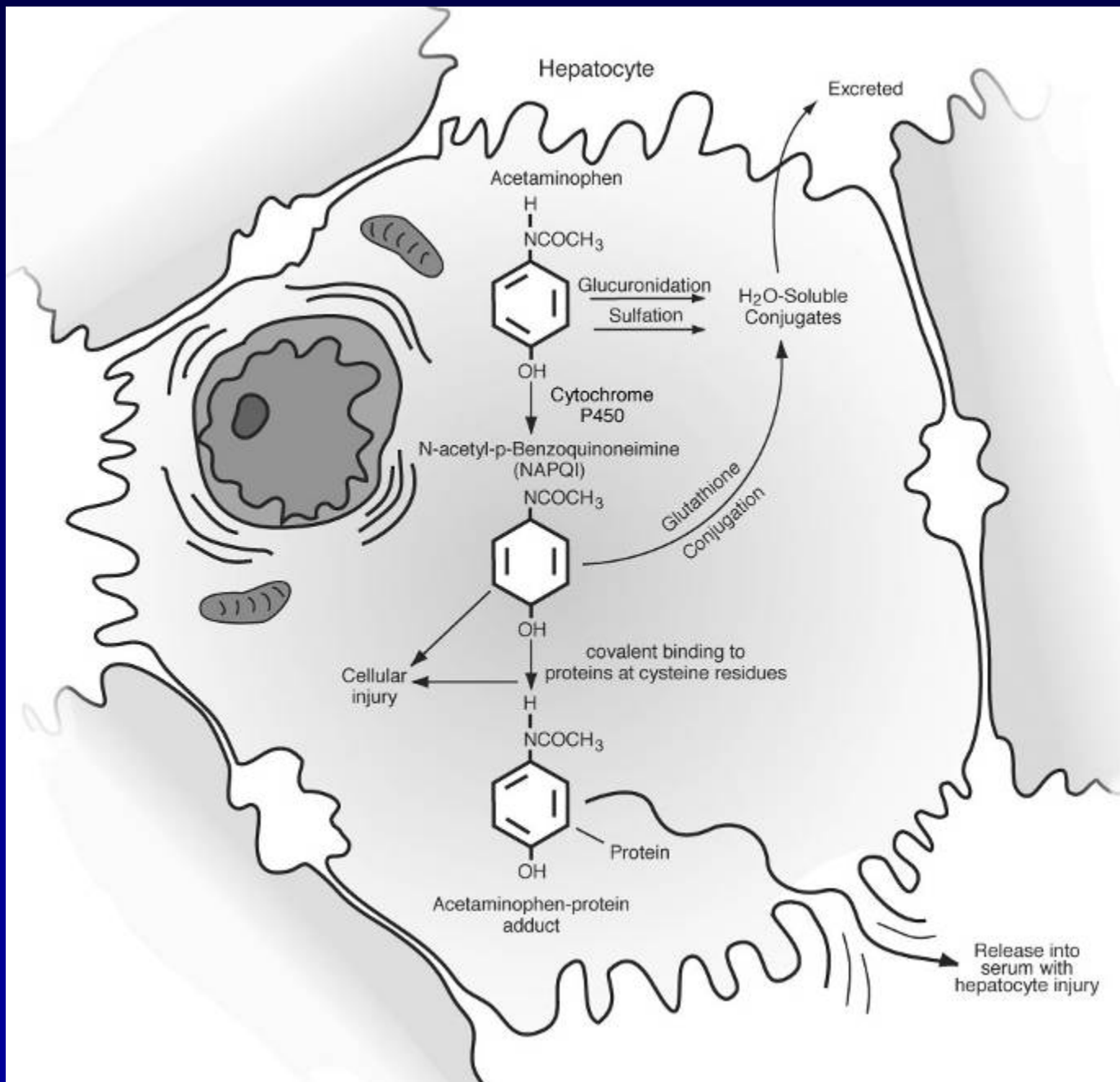
## *Why important?*

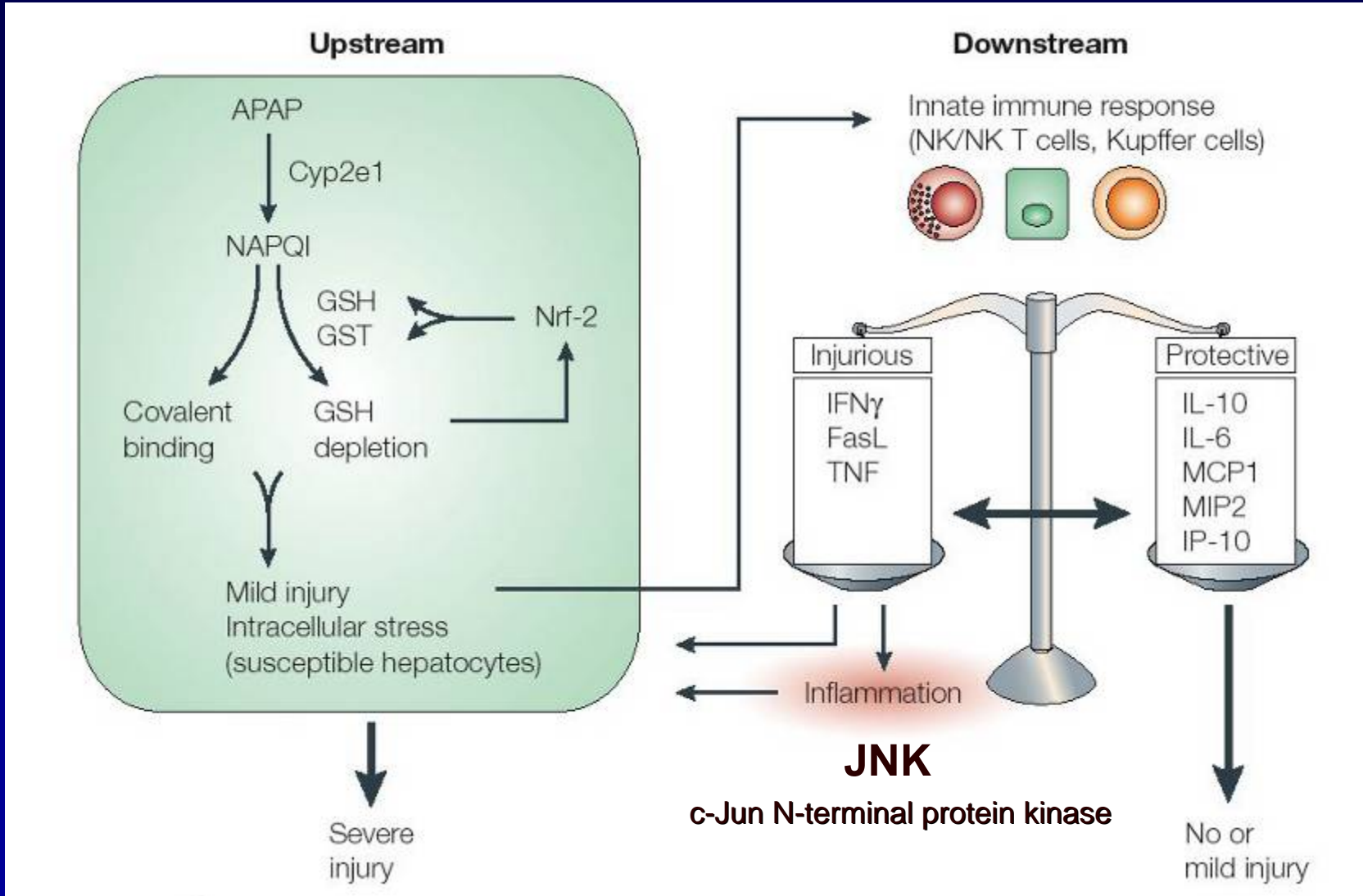
- Understanding the mechanisms underlying ART-related liver injury may lead to:
  - Effective prevention strategies
    - Pharmacogenomics
    - Rational drug design
  - Effective treatment
  - Improved tools for diagnosis
    - Specific biomarkers
    - Causality assessment

# Mechanisms of ART Liver Injury

*Why important?*







# Challenges in ART Liver Injury

## - Causality Assessment -

- Multiple drugs by definition
  - At least 3 antiretroviral drugs
  - Other potentially toxic drugs
  - Complementary alternative medications
- Coinfection with HCV and HBV (HDV)
- ETOH/recreational drug abuse common
- Diabetes/dyslipidemia → steatosis/steatohepatitis
- Other infections
  - Bacterial: bacillary angiomatosis, mycobacterial
  - Viral: CMV, EBV
- Tumors – lymphoma, KS
- Dechallenge difficult to assess as all ART agents typically stopped together

# Challenges in ART Liver Disease

## *Non-antiretroviral Drugs*

### Hepatocellular / Mixed

- INH
- PZA
- Rifampin
- Phenytoin
- Antifungals (azoles)
- Statins
- Antidepressants
- Antipsychotics

### Cholestatic

- TMP/sulfamethoxazole
- AMP/clavulanic acid
- Macrolides
- Anabolic steroids

# Challenges in ART Liver Disease

## - Study Design -

- Conflicting results from numerous studies reflects multiple factors
  - Definition of hepatotoxicity
  - Inadequate power
  - Retrospective, uncontrolled design
  - End point = ALT 5-10 × ULN; stop rules
  - No formal causality assessment
  - Heterogeneous populations
    - Age, gender, race
    - Prevalence of underlying liver disease (abnormal ALT)
    - CD4 count
    - Treatment history/duration/changes in ART

# Challenges in ART Liver Disease

## - Severe DILI -

- ALFSG (1998-2008): 3/1,321 (156 DILI) cases
  - 1 abacavir, 1 stavudine, 1 Atripla
- DILIN (2004-8): 15/459 cases
  - 2 nevirapine, 6 “ART”
- Swedish ADRAC (1970-2004): 0/784 cases
  - Bjornsson E, Olsson R. *Hepatology*. 2005;42:481-9
- Spanish DILD Group (1994-2004): 0/461 cases
  - Andrade RJ, et al. *Gastroenterology*. 2005;129:512-21
- WHO database (1968-2003): 303/4690 cases
  - Bjornsson E, Olsson R. *Dig Liver Dis*. 2006;38:33-8
  - “Interpreted with caution as causality assessment could not be performed”

# Challenges in ART Liver Disease

## - Severe DILI -

- Puoti M, et al. *J Acquir Immune Defic Syndr.* 2003;32:259-67
  - 26/755 patients prescribed new ART developed ALT  $10 \times$  ULN (or 5 x baseline if abnormal) - 4.2/100 person-years
  - Only 1 of 26 not HCV+ and all had low baseline CD4
  - 7/26 (26%) developed liver failure 3-25 days s/p ALT peak and all died - incidence of 1.1/100 person-years of ART
  - Authors felt IR played a major role in pathogenesis of liver injury
    - Correlation between CD4 and ALT increases
    - Histology (16 patients) c/with chronic hepatitis
    - Lack of rechallenge injury in >50%
    - Effect of anti-HCV Rx

# Challenges in ART Liver Disease

## - Severe DILI -

- Clark JJ, et al. *J Hepatol.* 2002;36:295-301
  - 6/375 patients with sudden-onset ALF admitted October 1997 – April 2000
  - None had evidence of HCV or active HBV infection; most had asymptomatic HIV
  - 5/6 died despite intensive therapy
  - Pathology c/w mitochondrial toxicity in 1 patient (on D4T and DDI) and confluent necrosis with little inflammation in the other 5
  - Authors questioned utility of monitoring

# ART Liver Disease

## - Mechanisms -

- Multiple potential mechanisms of liver injury in the setting of ART
  - “Direct toxicity”
  - Hypersensitivity (NNRTIs)
  - Mitochondrial toxicity (NRTIs)
  - Immune reconstitution (with HCV, HBV coinfection)
  - “Interference” (with HCV, HBV coinfection)
  - Insulin resistance leading to NAFLD

# ART Liver Disease

## - Mechanisms -

- Idiosyncratic changes in drug metabolism, cytoprotection, etc.
- Major interplay between drugs, host, viruses, and environment
  - Drugs and environment alter host interaction w/viruses
  - Viruses may interact with host and alter metabolism/response to drugs
- HIV-HCV-HBV reciprocal interference
  - ↓HIV → ↑HCV → liver injury

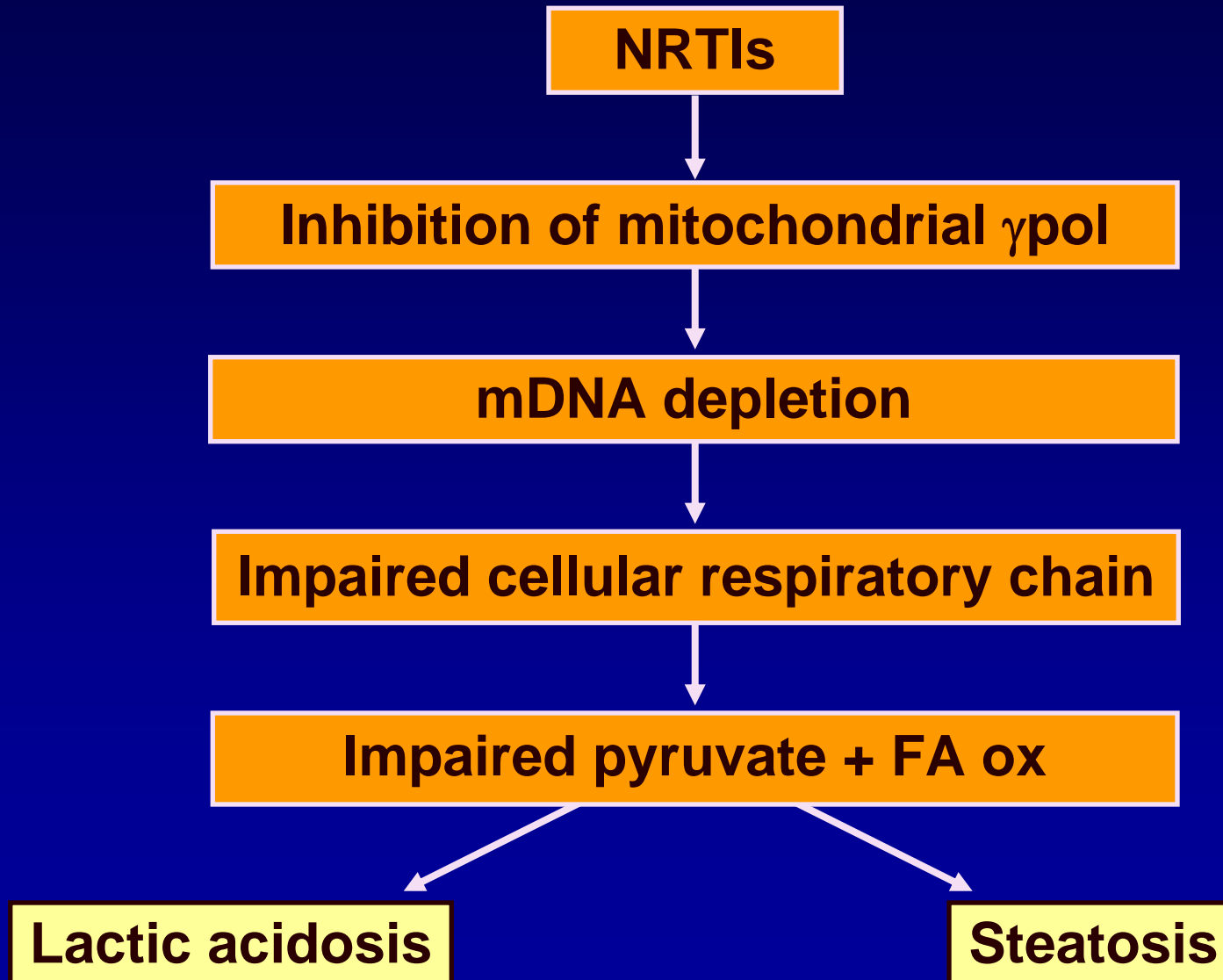
# Mitochondrial Toxicity

- Frequently associated with peripheral lipodystrophy
- Insidious onset of signs and symptoms
  - Fatigue, N/V, anorexia, weight loss, weakness
  - Lactic acidosis, hypoglycemia, increased NH<sub>3</sub>, CPK, amylase/lipase but often only modestly increased ALT
  - Microvesicular fat with focal necrosis, fibrosis, cholestasis, BD proliferation, Mallory bodies
- Risk related to cumulative exposure NRTIs?
- Relative inhibition of mitochondrial  $\gamma$ pol:

**Zalcitabine > didanosine\* > stavudine\* >  
zidovudine\* > lamivudine = abacavir = tenofovir**

\*Clinically most often associated with mitochondrial toxicity.

# Mitochondrial Toxicity



# Mitochondrial Toxicity

- Mitochondrial DNA depletion results in:
  - Respiratory chain dysfunction with impaired pyruvate and FA oxidation-----> steatosis
  - Anerobic glycolysis -----> lactate production
  - Increased intracellular ROS
    - Lipid peroxidation of accumulated FA
    - Release of proinflammatory cytokines
    - DNA damage
    - Release of cytochrome C-----> PCD
  - Decrease in dihydroorotat-ubiquinone oxidoreductase (DHODH)-----> decrease in *de novo* synthesis of pyrimidine nucleosides

# Mitochondrial Toxicity

- Inhibition of mitochondrial DNA synthesis → anaerobic metabolism and increased lactate production
  - Normal plasma [lactate] = 1-2 mmol/L
  - Acidosis w/ plasma [lactate] = 5 mmol/L
  - Chronic compensated increased plasma lactate common
- Risk factors for severe mitochondrial toxicity
  - Female gender
  - Race
  - Underlying liver disease
  - Coinfection HCV, HBV
  - Pregnancy
  - Obesity
- Utility of measuring mtDNA deletions in peripheral blood?

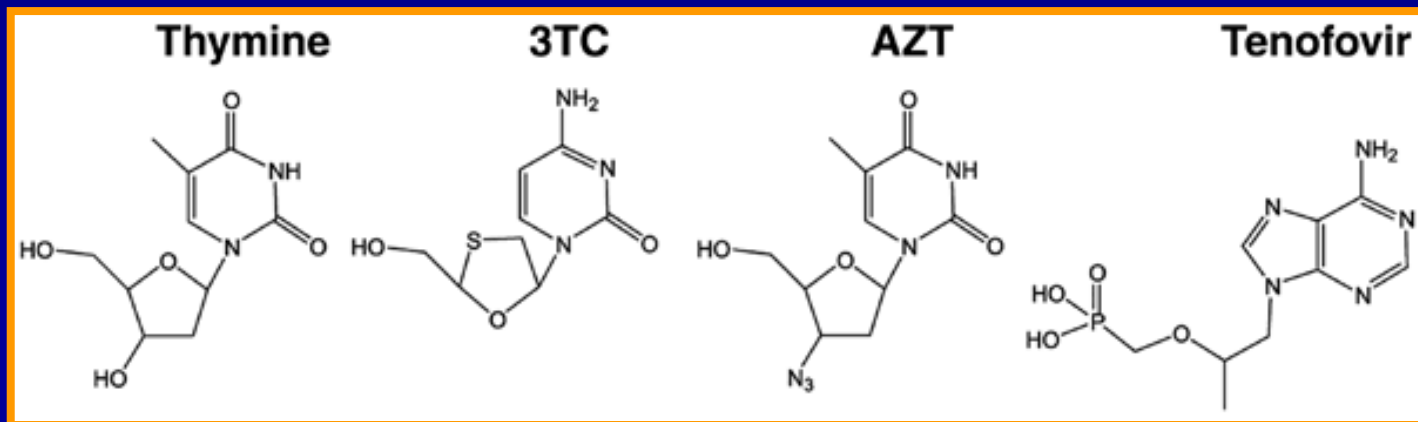
# Mitochondrial Toxicity

- Measurement of mDNA/nDNA in PBCs
  - Real time quantitative PCR assay
  - 3 cohorts studied
    - HIV negative (n = 27)
    - HIV positive, ART naive (n = 47)
    - HIV positive, ART c/b symptomatic hyperlactatemia (n = 8)
  - Results:
    - Mito/nuc lower in HIV+ versus HIV- subjects
    - Mito/nuc lower in symptomatic versus asymptomatic subjects
    - Mito/nuc increased when ART discontinued
    - Mito/nuc decreased before serum lactate levels increased
  - Subsequent cross-sectional study demonstrated significantly higher mito/nuc in subjects on a stavudine-sparing regimen

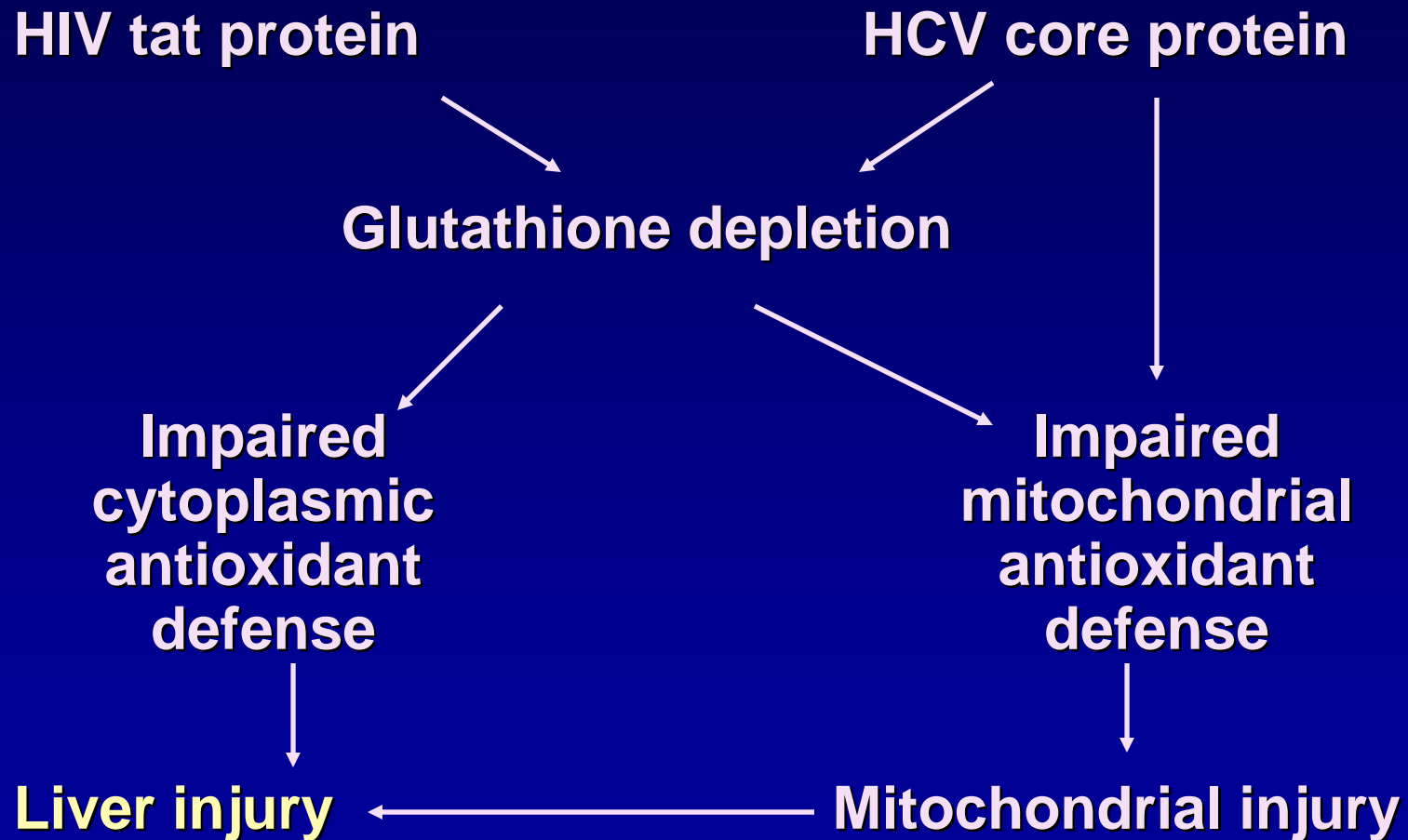
Cote HC, et al. *N Engl J Med.* 2002;346:811-20.

# Mitochondrial Toxicity

- Stavudine and didanosine are associated with highest risk of mitochondrial toxicity
- Patients (114) with d4T-associated mitochondrial toxicity (lipoatrophy or hyperlactatemia) - tolerated either ABC or AZT  $\times$  1 year (Lonergan, et al. *Antiviral Ther.* 2002)
- Risk of chronic liver disease?

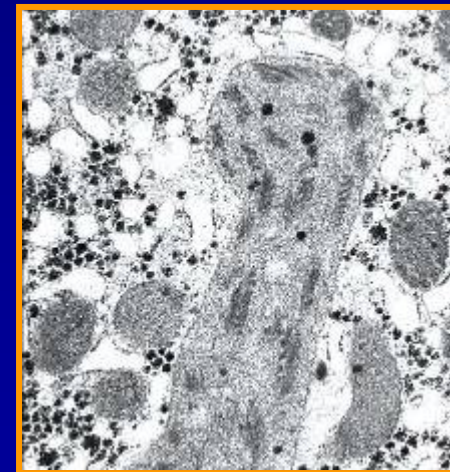
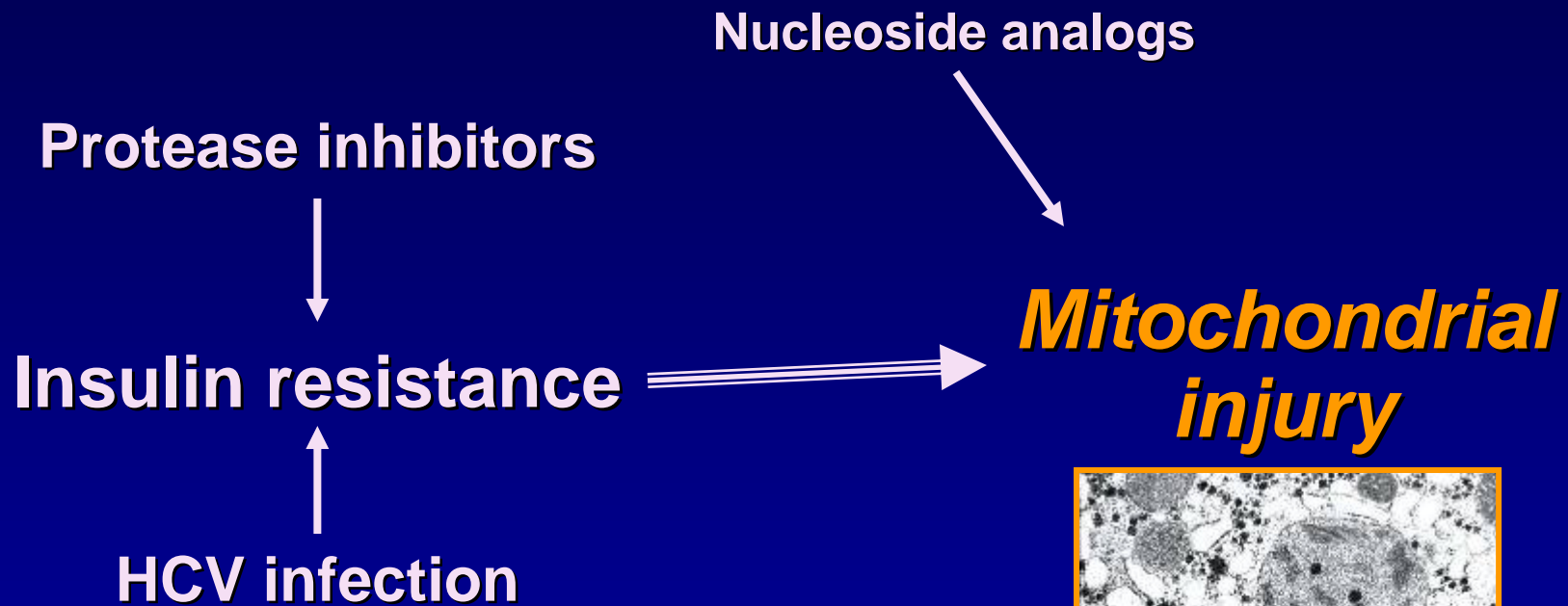


# Mitochondrial Toxicity



Modified from Bonacini M. *Clin Infect Dis.* 2004;38(suppl 2):S104-8.

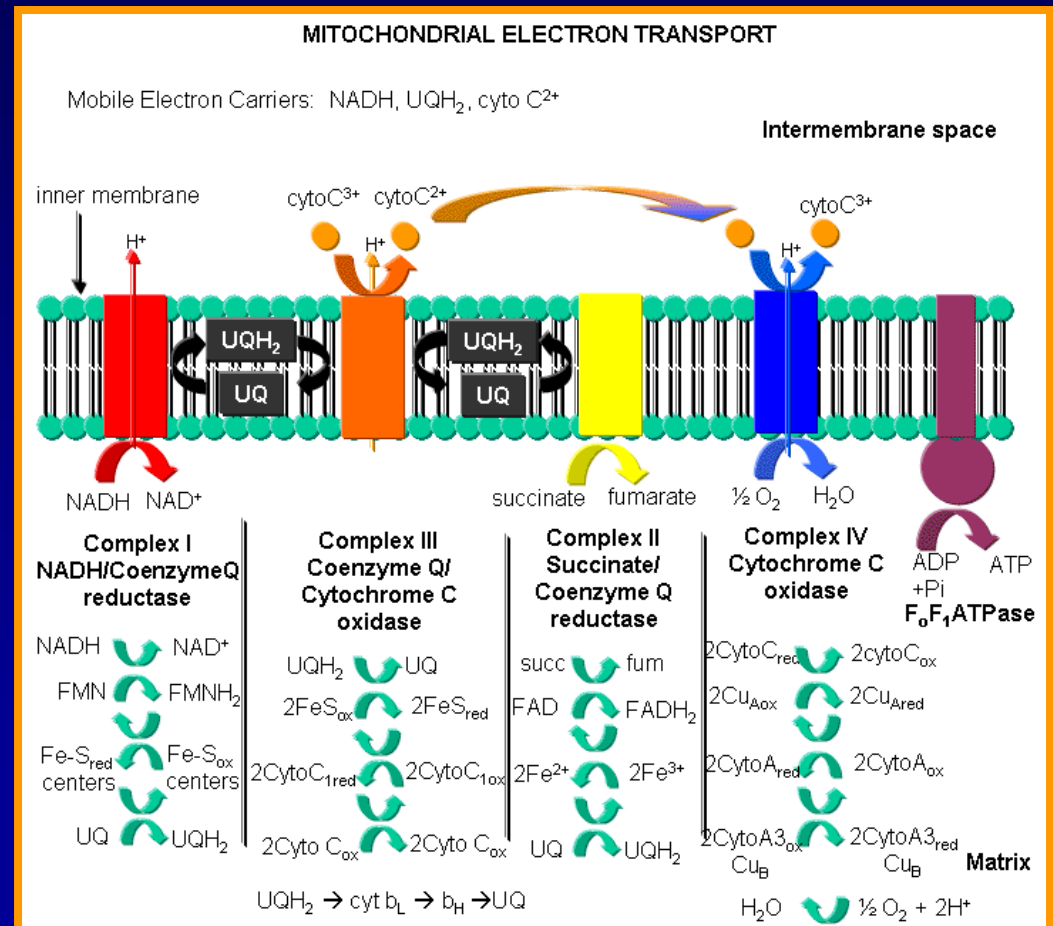
# Insulin Resistance



# Mitochondrial Injury

## - Treatment -

- Vitamin B complex forte BID
  - Thiamine 50 mg
  - Riboflavin 10 mg
  - Nicotinamide 100 mg
  - Pyridoxine 10 mg
  - Dexpanthenol 10 mgL-
  - carnitine 1000 mg IV BID
- Co-enzyme-Q
- Lipoic acid
- Folic acid
- Other antioxidants
- Uridine



# Immune Reconstitution

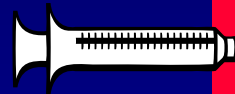
- Typically occurs in coinfecting patients with
  - Low baseline CD4
  - High baseline HIV
  - Rapid and robust (>50) rise in CD4 s/p ART
- +/- change in HCV titer
- Histology – severe hepatitis
- ALT associated with IR usually resolves
  - If patient does not have rise in CD4 count s/p ART - worse liver injury at 12 months (Acoti, et al. *JAIDS*. 2002)
- Markers of HCV-specific immune response (eg, HCV core-specific IgG), T-cell activation and inflammation correlate with liver injury (Stone SF, et al. *J Infect Dis*. 2002;186:1498-502.)

# Chisari HBV Envelope Transgenic Mouse Model

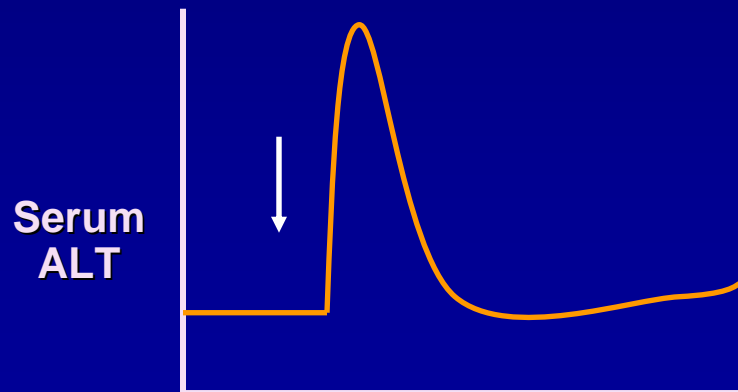
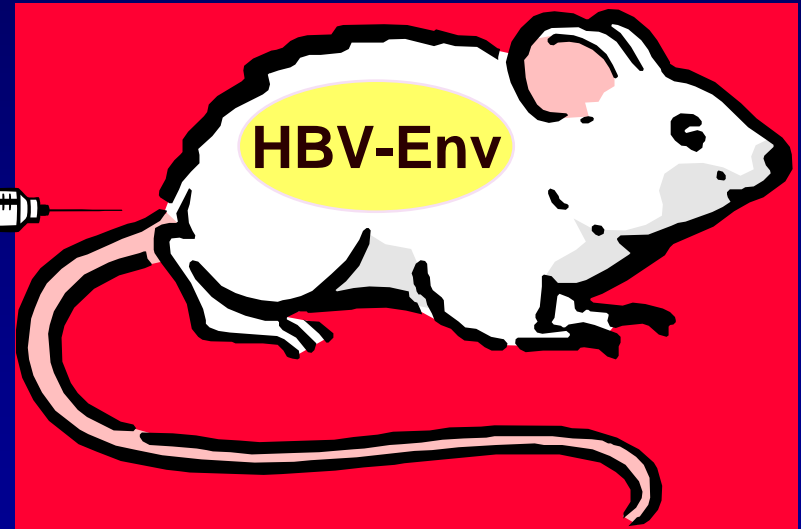
Isolate splenocytes from HBV immunized mice



Inject CD8 T cells specific for HBV Env

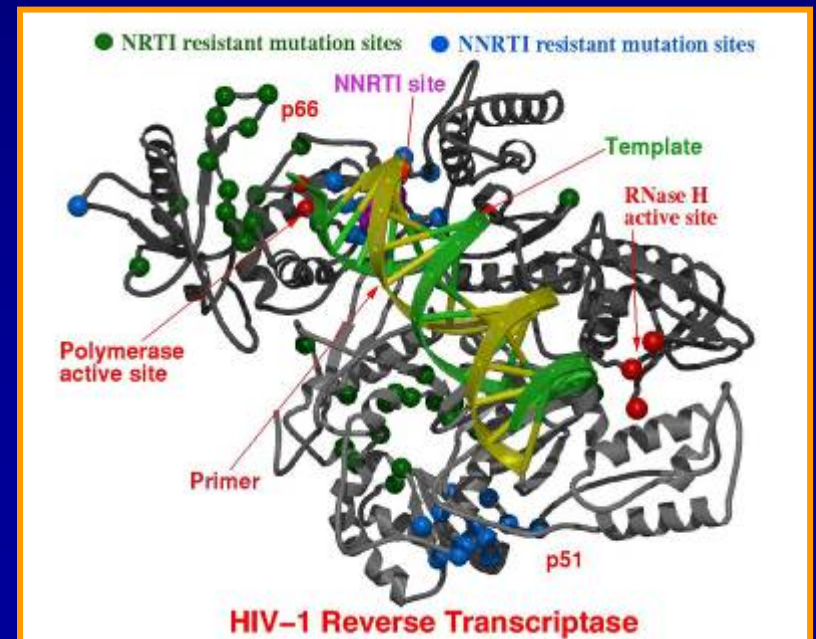


HBV-envelope transgenic mice



# Non-nucleoside Reverse Transcriptase Inhibitors

- Conflicting data regarding relative toxicity of nevirapine (NVP) c/w other NNRTIs (eg, efavirenz)
- Risk factors for liver injury w/ NVP
  - Baseline CD4 >350
  - Elevated baseline serum ALT
  - HCV or HBV coinfection
  - ? Prolonged ART
  - ? ddT

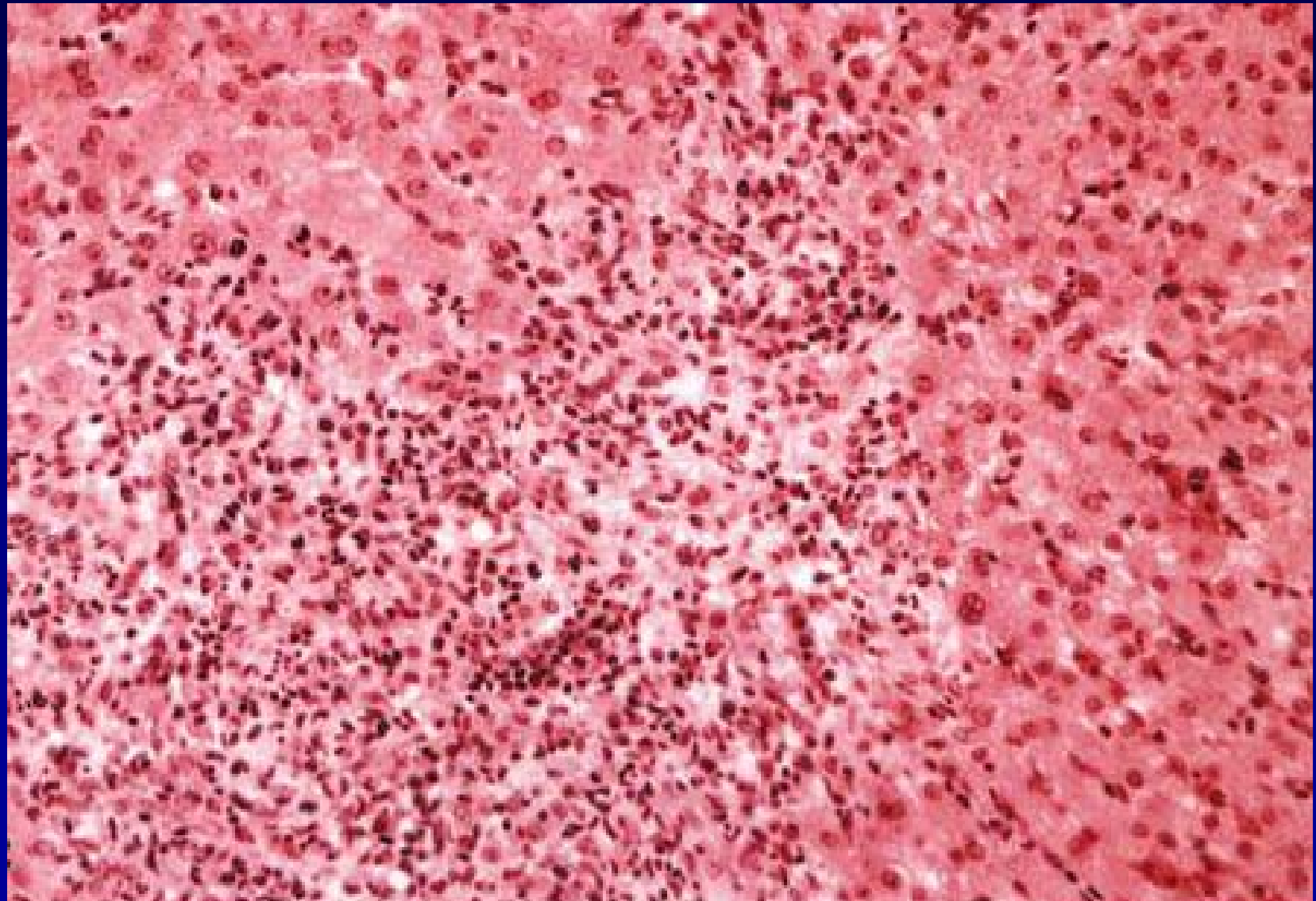


# Non-nucleoside Reverse Transcriptase Inhibitors

- 2 distinct types of NVP-related liver injury
  - Early (within 6-8 weeks) = hypersensitivity
    - More common in black females
    - Relatively high CD4 counts appear to increase risk
    - HCV coinfection does not appear to increase risk
  - Late = idiosyncratic
    - More common form of NVP liver injury
    - Exacerbated by underlying hepatitis
- Dose dependent hepatotoxicity
  - Serum [nevirapine] and risk of liver injury unclear

# Non-nucleoside Reverse Transcriptase Inhibitors

- Rash – 32% to 48% of patients on nevirapine
- Review of all RCT of nevirapine (1732 cases, 1912 controls)
  - 5% symptomatic hepatic events; 3.5 RR
  - RR of hypersensitivity syndrome = 11
  - ALF rare (0.1%)
- “Dear Doctor” letters mailed November 2002
  - “Continued reports of severe, life threatening and, in some cases, fatal hepatotoxicity”
  - Suggested close monitoring during first weeks of Rx, 2 weeks s/p dose escalation, and periodically thereafter
- HIV-seronegative patients high risk for liver injury associated with nevirapine





a) 1959, original SNS model said that lymphocytes are activated by recognition of foreign things.



b) 1969, 1st modification: B cells die when they see antigen (signal one) unless rescued by help (signal two).

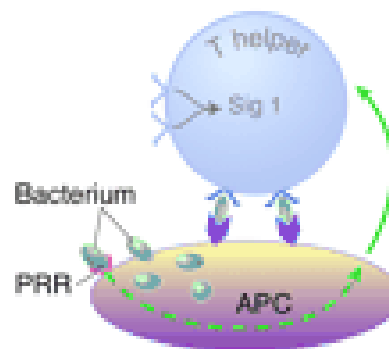
Sig 2 (Help)



c) 1975, 2nd modification: T helper cells die when they see antigen unless rescued by co-stimulation (signal two) from APCs.

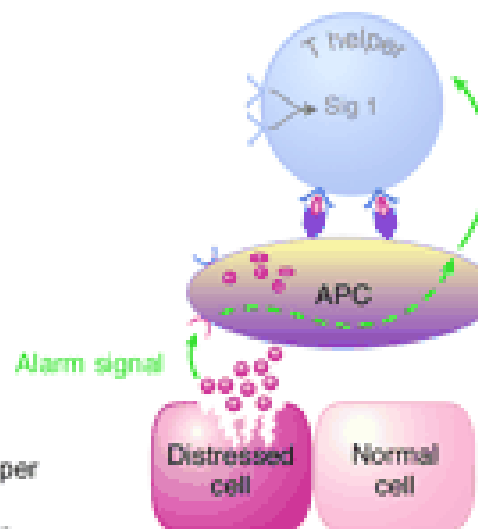
Sig 2 (Help)

Sig 2 (Co-stimulation)



d) 1989, 3rd modification (INS): APCs do not co-stimulate unless activated via PRRs (receptors for evolutionarily distant infectious non-self).

Sig 2 (Co-stimulation)



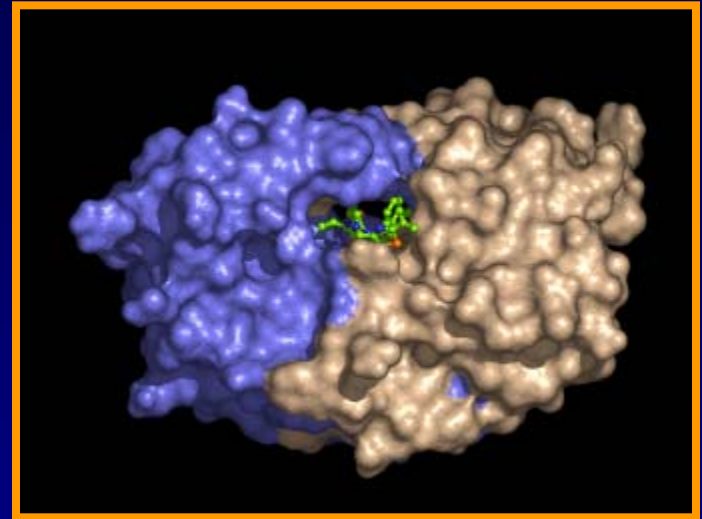
e) 1994, 4th modification (Danger model; major change) APCs are activated by endogenous cellular alarm signals from distressed or injured cells.

Sig 2 (Co-stimulation)

*A History of Immunological Models*  
- Matzinger P. *Science*. 2002

# Protease Inhibitors

- Some studies suggest ritonavir (RTV) is more hepatotoxic than other PIs
  - Sulkowski MS, et al. *JAMA*. 2000;283:74-80
    - 31 (10.4%) of 298 HIV patients develop severe liver injury
    - Patients on a full dose (600 BID) RTV-based regimen had highest risk (~30%)
    - ? Secondary to RTV inhibition of P450 (3A) -> increasing serum [ ] of other drugs
  - Not confirmed by some other studies
    - Generally, some PIs (saquinavir, nelfinavir, lopinavir, amprenavir) are regarded as less liver toxic than others (ritonavir, indinavir, tipranavir)



# Pharmacogenetics of Antiretroviral Therapy

- HLA haplotype and abacavir hypersensitivity
  - More common in whites than blacks
  - More common in family members
  - HLA-B\*5701, HLA-DR7, HLA-DQ3 present in 72% cases and 2% controls → 100% PPV + 97% NPV in a Western Australian population (Mallal, et al. *Lancet*. 2000)
  - Retrospective study of 85 cases, 115 controls reported much lower sensitivity (55%) OF HLA-B\*5701
    - None of the black cases had this allele
- PI-induced hyperbilirubinemia
  - Indinavir, atazanavir → unconjugated hyper bili (6%–40%)
  - *UGT1A1* 7/7 highly predictive of bilirubin >2.5 mg/dL
  - No clinical toxicity

# ART Liver Injury

## - Treatment -

- Standard treatment for DILI -> d/c offending agent – challenging with ART
  - ART is essential
  - HIV resistance rapidly develops to single/double agents
  - HIV resistance may dictate drug changes
  - Concern re: “class effect”
- Rule out other treatable causes of liver injury
  - HBV reactivation
  - Other viruses, toxins (APAP, ETOH, illicit drugs)
- ? Role of HCV treatment
- ? Role of steroids, antihistamines, reducing steatosis
- ? Role of liver transplant

# ART Liver Disease

## - Future Directions -

- Well-designed studies of liver injury in setting of ART
  - Drug Induced Liver Injury (DILIN)
  - Acute Liver Failure Study Group (ALFSG)
  - AIDS Clinical Trial Group (ACTG)
- More robust causality instruments
  - Bayesian approach?
- Bioassays for liver injury from drugs
  - APAP adduct assay
  - Mitochondrial DNA injury
- Pharmacogenomics