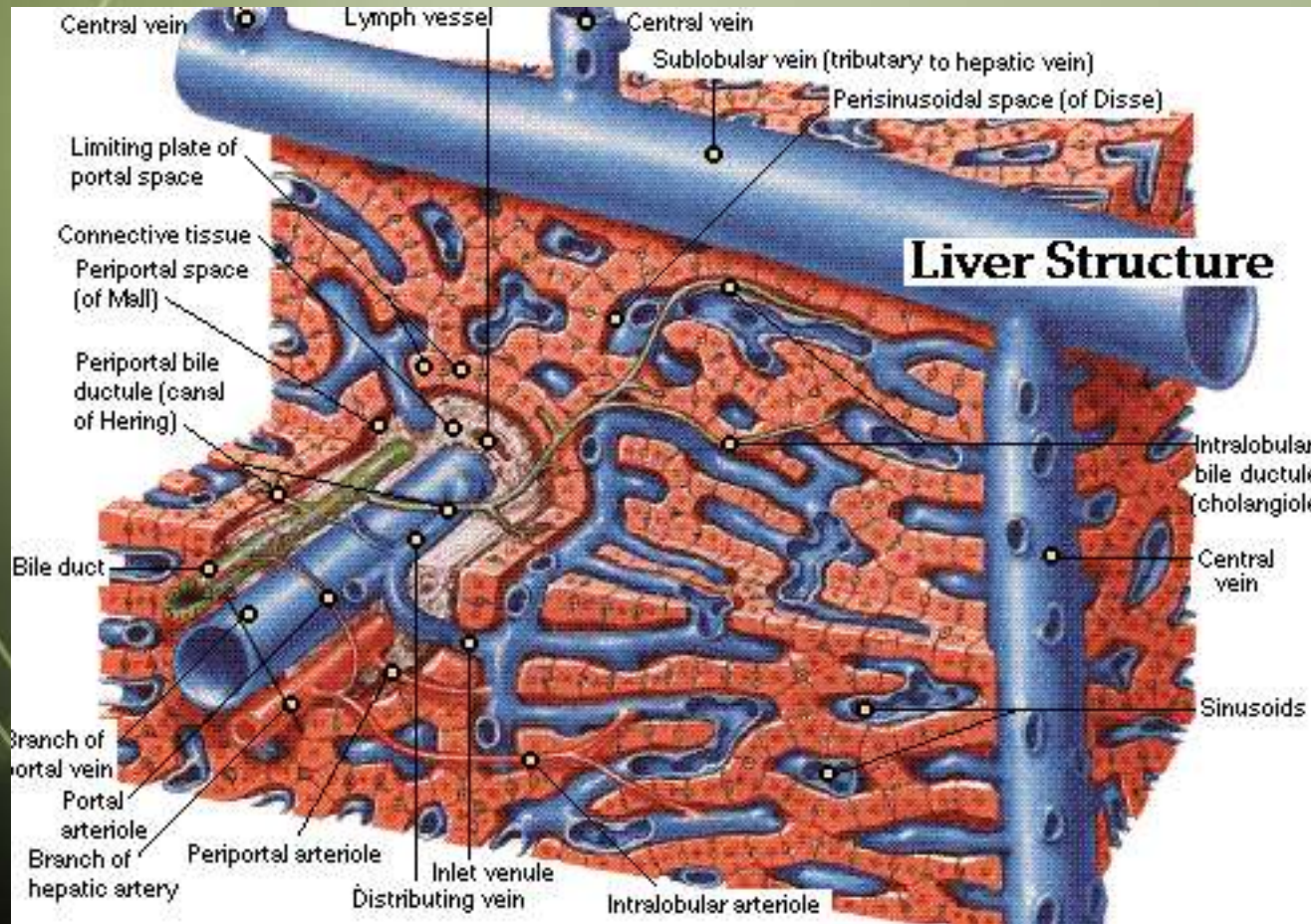


What I will say today:

- 1. Primary biliary cirrhosis (cholangitis) or PBC is caused by bad genes and bad luck*
- 2. And especially by chemicals and mimic bacteria*

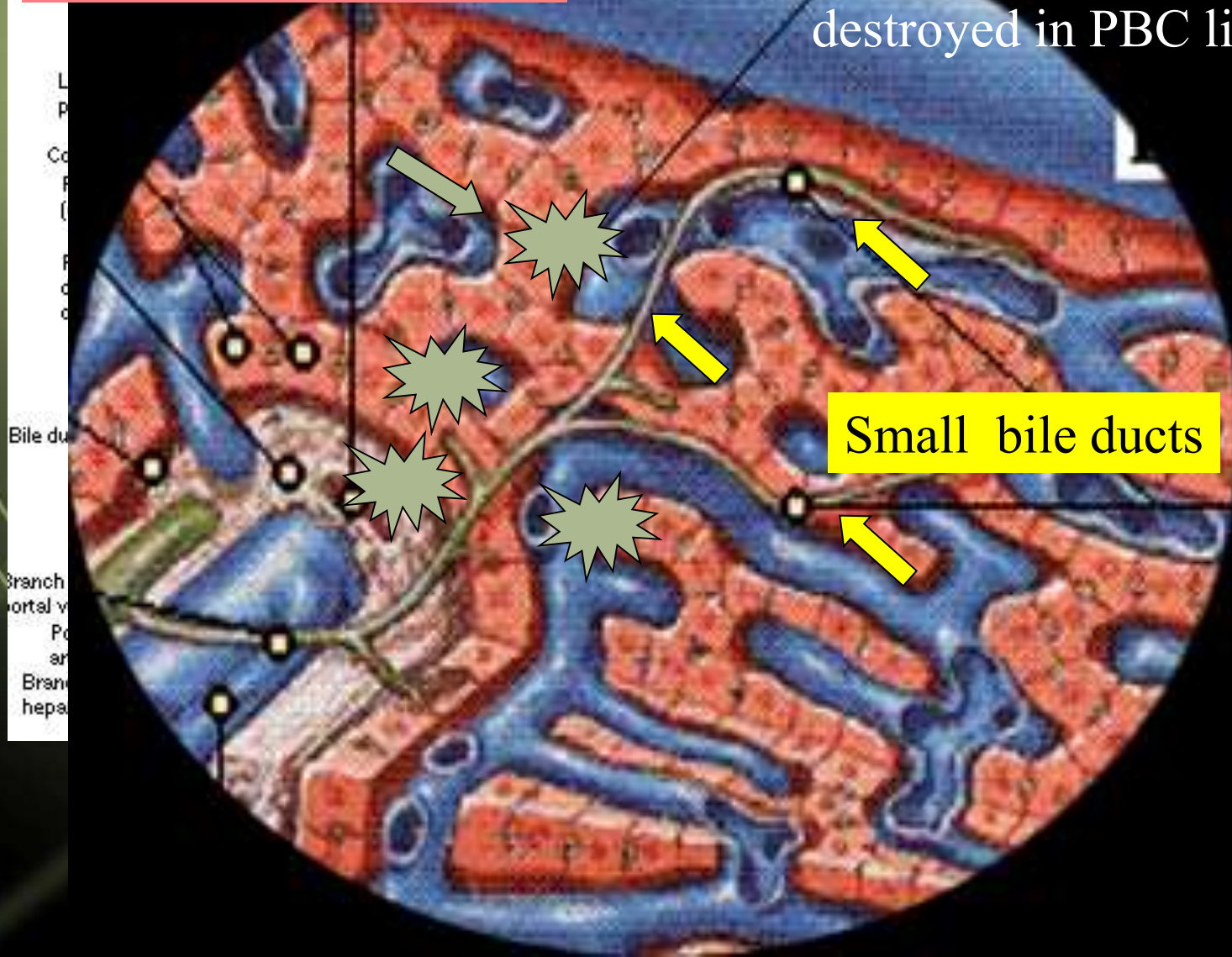
Liver Nodule Blow -Up



Liver Nodule Blow -Up

Lymphocyte infiltration

Small bile ducts are specifically destroyed in PBC livers



Small bile ducts

Etiology???

Individual susceptibility

Genetics

There is an extremely high concordance in monozygotic twins (0.63 pairwise) with PBC. (RA= 0.15)

The risk factor for developing PBC in a first degree relative is 100-800 fold more common and the onset of disease in relatives is often within a few years of each other's diagnosis
Immune genetic component(s) is still unknown.

Environmental Factors

Clustering of PBC cases

Xenobiotics--- can break tolerance and induce AMA in animal studies.

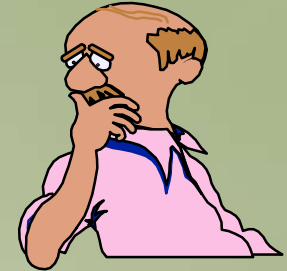
Infectious agents:

Bacteria-- Antigen mimics of PDC-E2

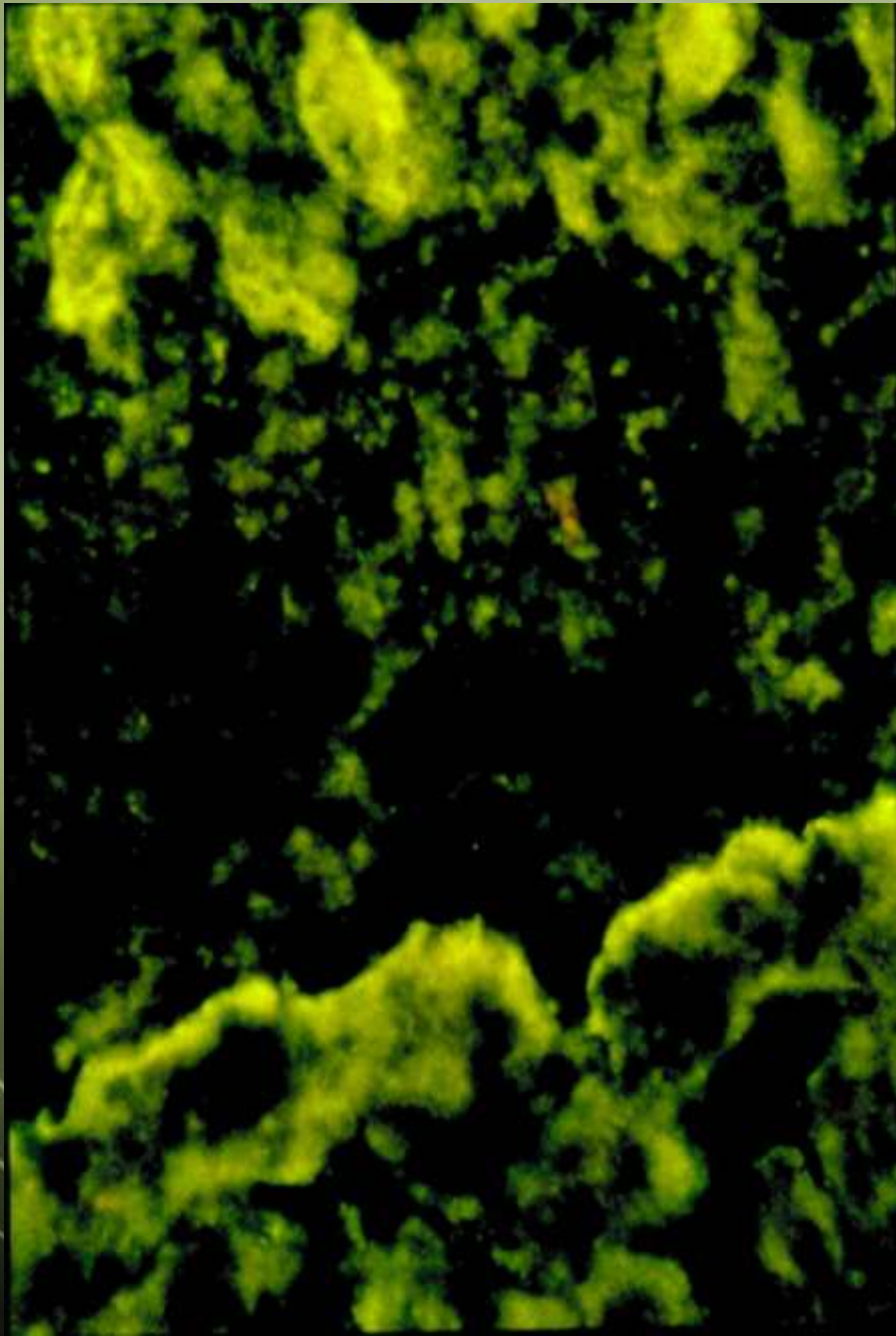
Virus?????

Key Questions in Primary Biliary Cirrhosis

???



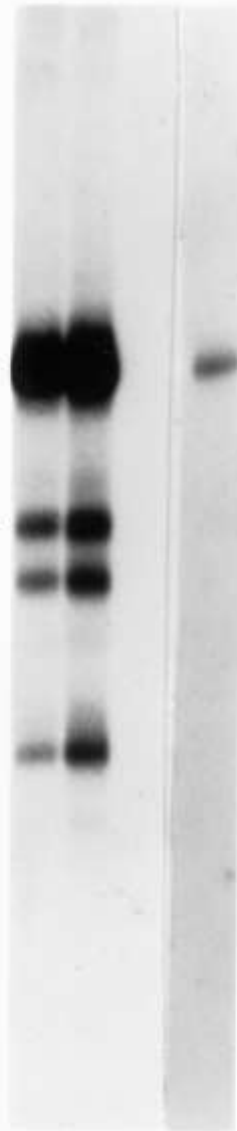
- How does the immune system initiate the immune response to self intracellular mitochondrial proteins ?
- All cells in the body have mitochondria. Then, why do only bile duct epithelial cells Become target of the self immune system ?
- Are antimitochondrial autoantibodies the primary or secondary immune response of this disease ?



A B C D

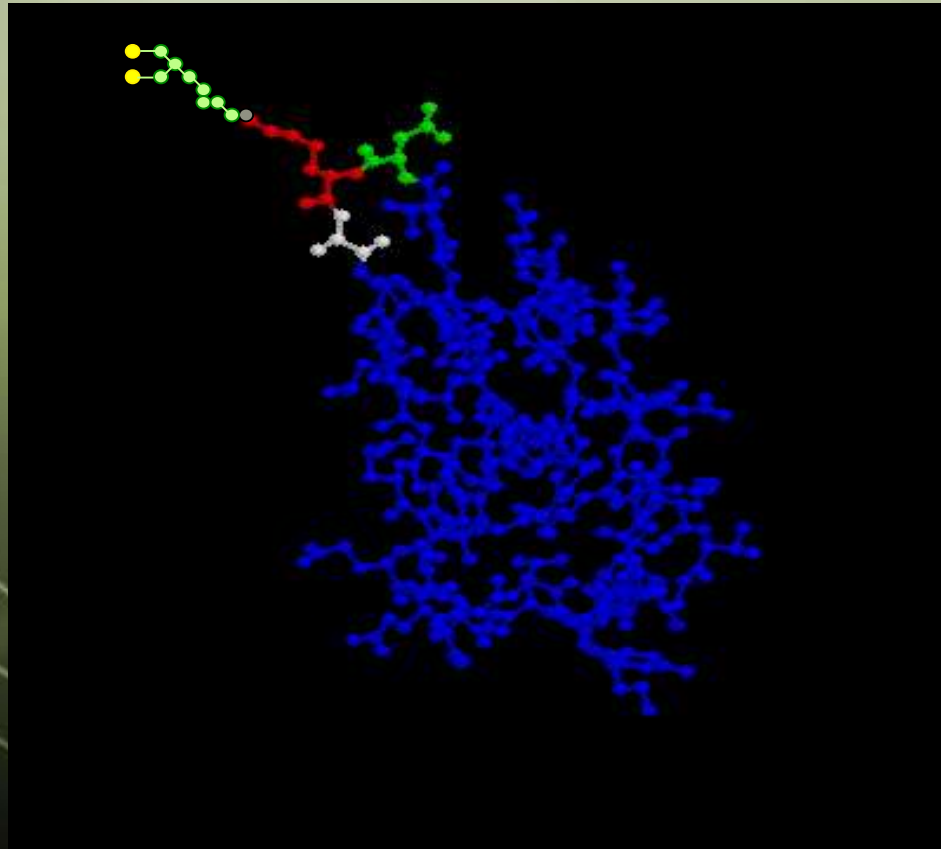
74—

52—



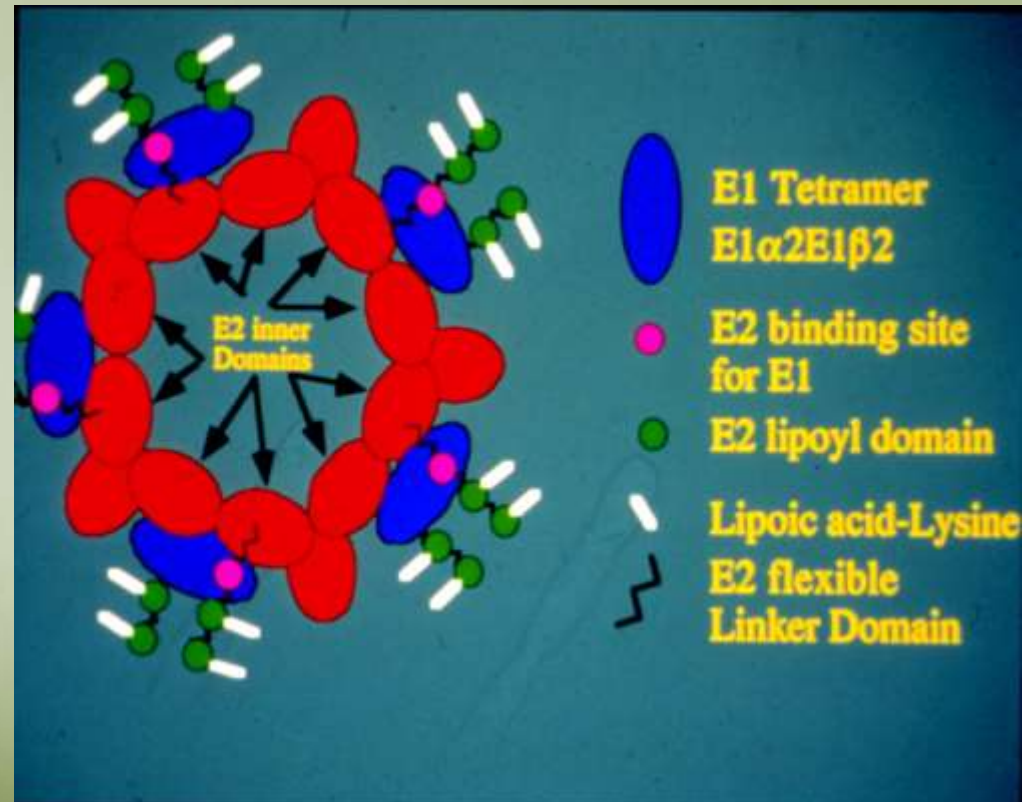
Introduction

30 years ago, Pyruvate dehydrogenase E-2 subunit (PDC-E2) was identified as the immunodominant mitochondrial autoantigen in Primary Biliary Cirrhosis (PBC)



Mitochondrial Antigens of PBC

- PDC-E2 (74 kD)
- Protein X (56 kD)
- BCOADC-E2 (52 kD)
- OGDC-E2 (48 kD)
- PDC-E1 α (39 kD)



All members of Pyruvate Dehydrogenase Complex

Result

- ⊕ If you have a positive AMA with recombinant autoantigens you better have good medical insurance

Over The years

Define
Epitopes

Environmental
(Xenobiotic Data)

Mouse
Models

1987

2019

Identified
Autoreactive
CD 4 and Cd8

Epidemiological
data

CLINICAL PRESENTATIONS

Convenient truths.

Primary biliary cirrhosis (PBC) is a autoimmune liver disease with a high female predilection.



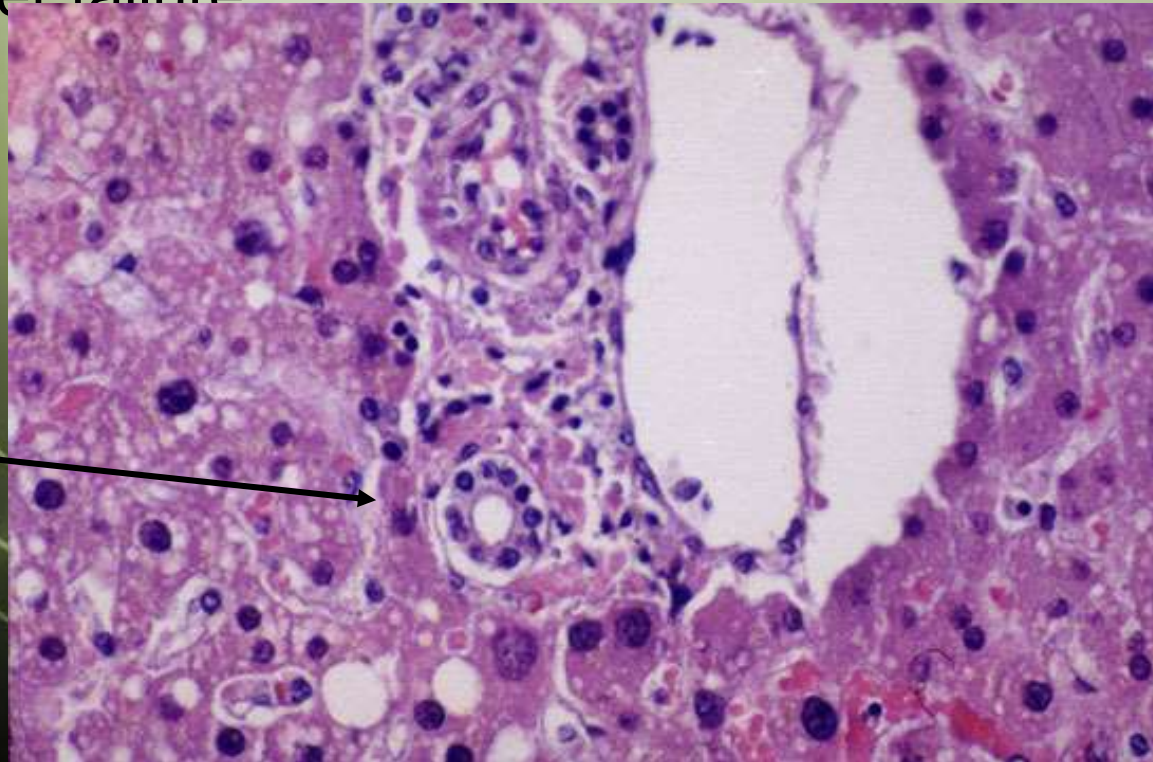
CLINICAL PRESENTATIONS



Convenient truths.

Characterized by progressive immune-mediated destruction of intrahepatic biliary ductules.

Resulting in decreased bile flow, "obstructive" liver functional indices, hepatic fibrosis and cirrhosis, and eventual liver failure



Bile Duct

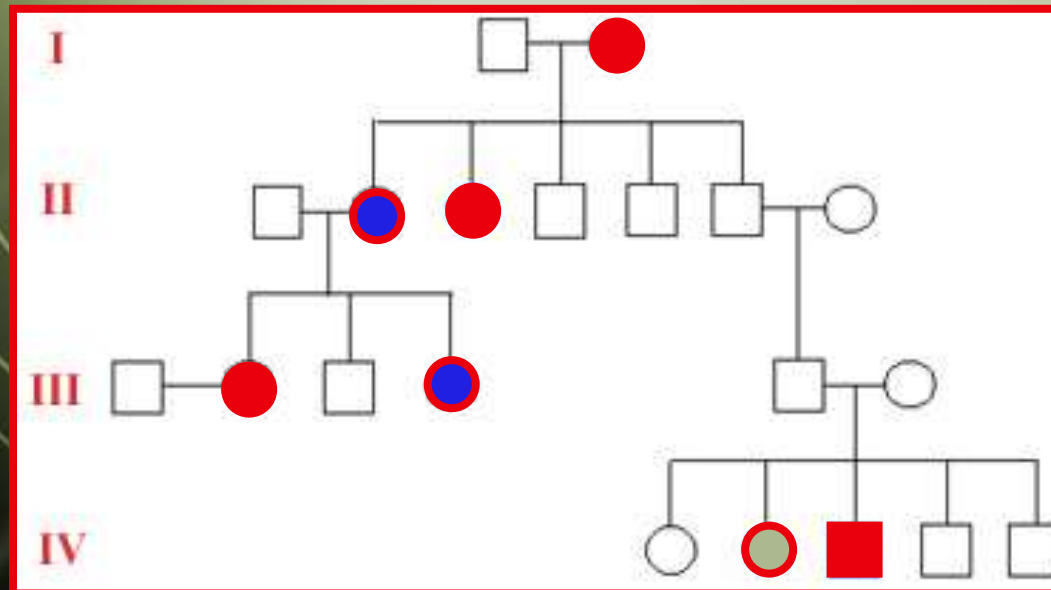


CLINICAL PRESENTATIONS



Convenient truths.

PBC is clearly associated, within individuals and among family members, with other autoimmune diseases, either organ-specific or multisystem , reflecting the “clustering” so characteristic of autoimmunity .



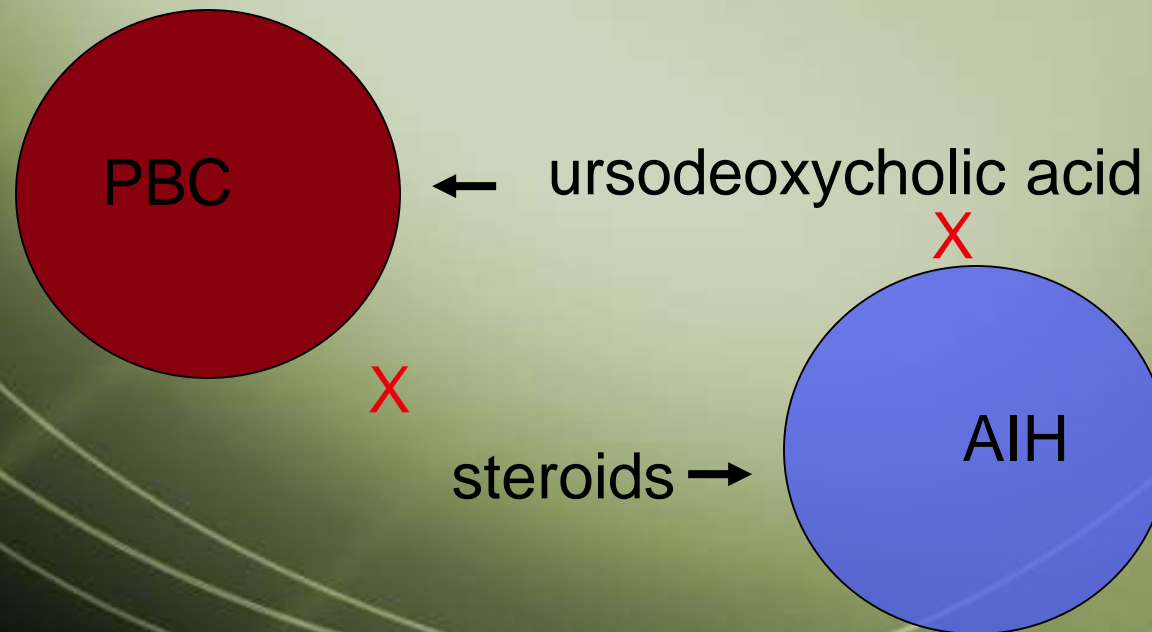
- Autoimmune diseases
- PBC

CLINICAL PRESENTATIONS

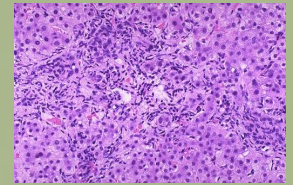


Inconvenient Truths

Additionally PBC is not highly responsive to conventional immunosuppressive drugs yet therapeutic benefit (unique to PBC) is conferred by ursodeoxycholic acid that is not regarded as an immunomodulatory or anti-inflammatory agent.

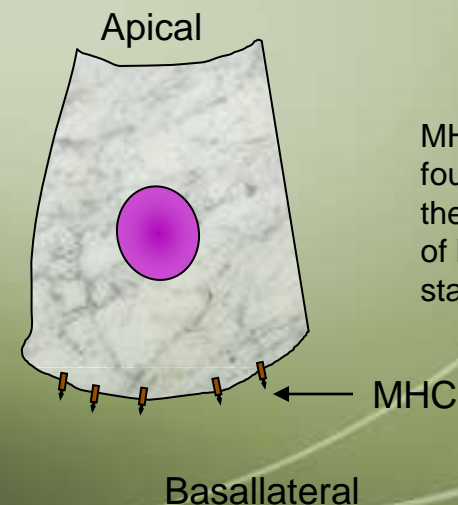
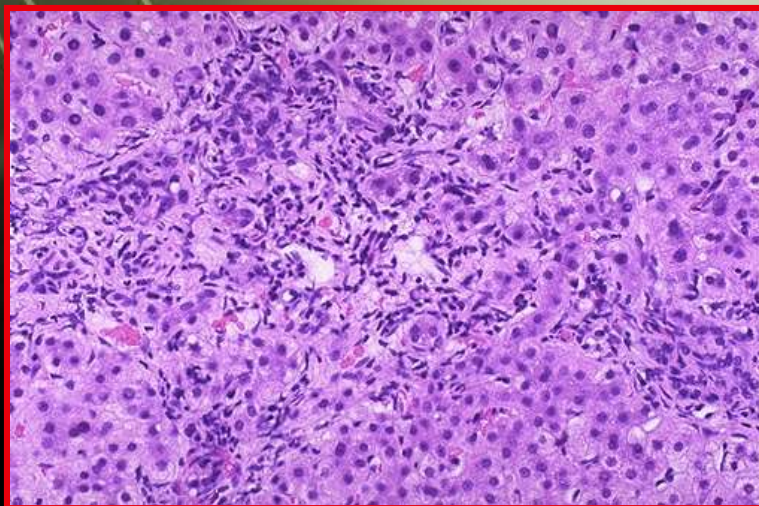


PATHOLOGIC FEATURES



Convenient truths.

The specificity of pathological changes localized to the **bile ducts**, the presence of **lymphoid infiltration** in the portal tracts, and the readily detectable **expression of MHC antigens** on the biliary epithelium indicate that autoantigen-specific T cell responses are directed against biliary epithelial cells (BEC).

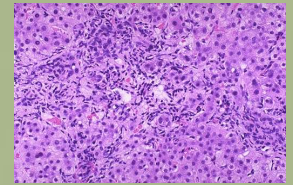


MHC molecules are found expressed on the basallateral side of BEC's in earlier stages of PBC

***Do autoreactive T cells
play a role
in the pathogenesis of
PBC?***



Dr. Shinji Shimoda

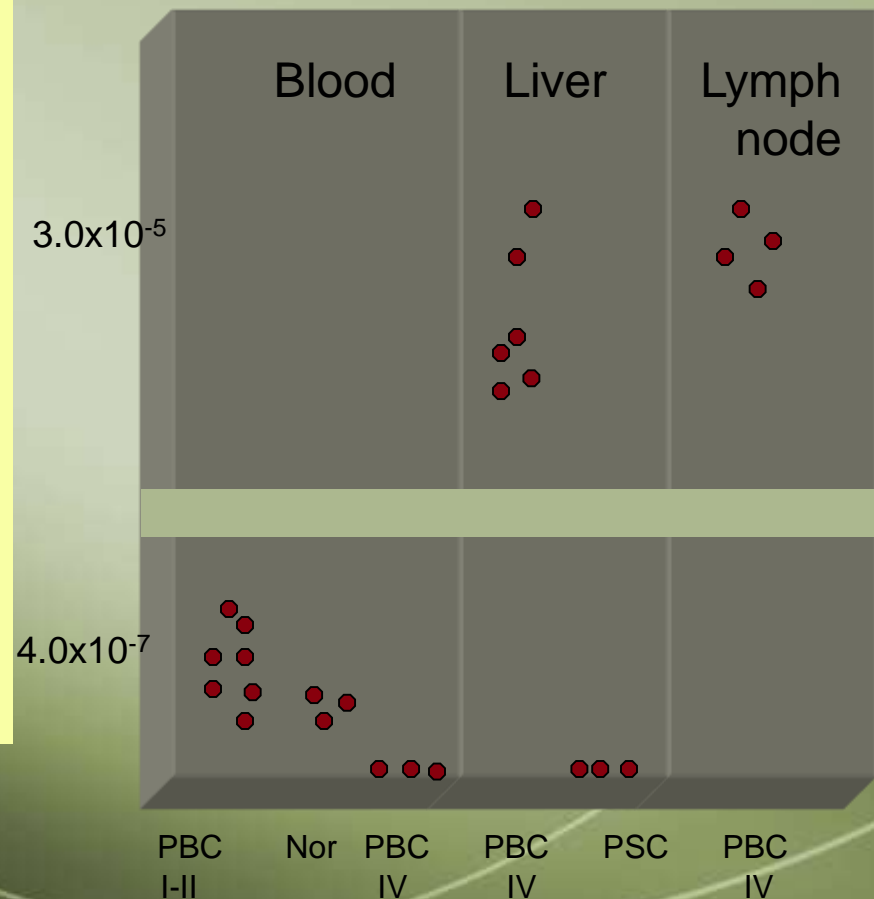


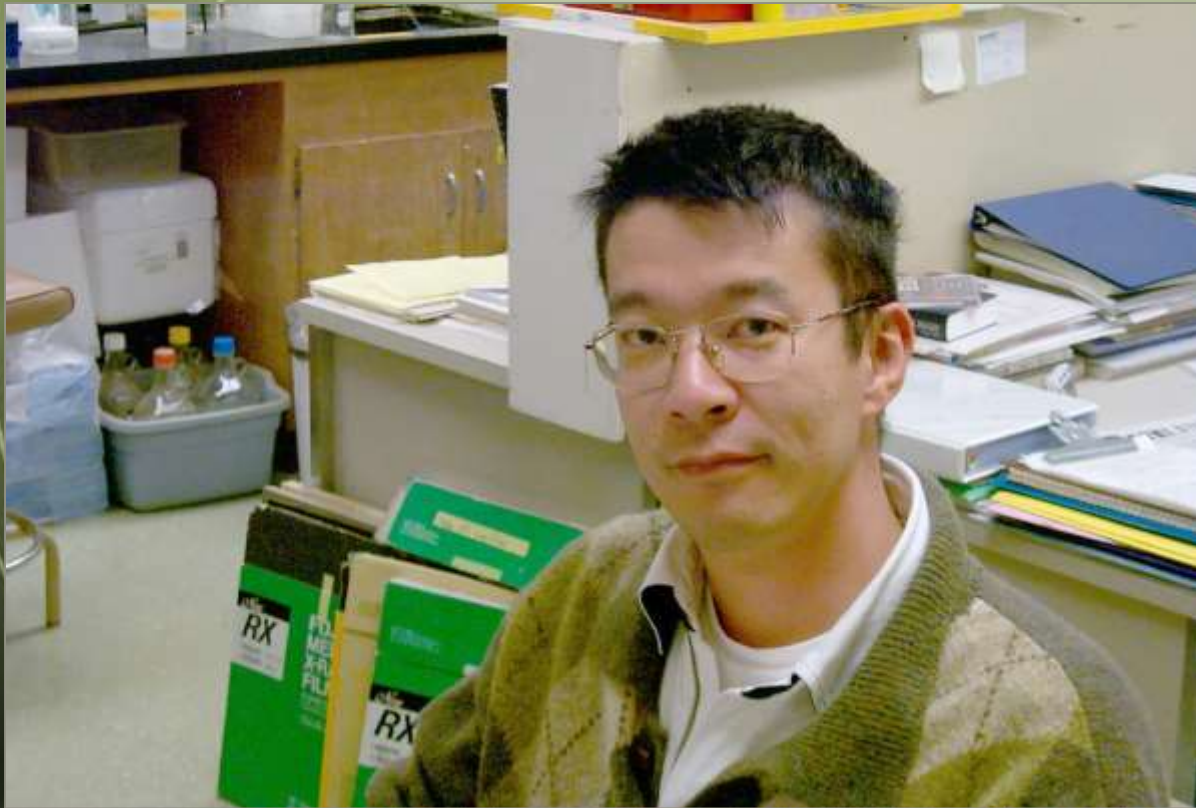
PATHOLOGIC FEATURES

Convenient truths.

Our laboratory has accumulated substantial data suggesting that the destruction of biliary cells in PBC is mediated by liver-infiltrating autoreactive **CD4+ T cells** with specificity for the immunodominant PDC-E2 autoantigen, and MHC class II-restricted target epitopes have been mapped .

Reactive frequency of T cells to inner lipoyl peptide of PDC-E2





Dr. Hiroto Kita

Tetramer staining of PDC-E2 specific CD8 T cells after in vitro stimulation of PBMCs with PDC-E2₁₅₉₋₁₆₇

Sample	PDC-E2 ₁₅₉₋₁₆₇ tetramer ⁺ CD8 ⁺ cells (%)	Sample	PDC-E2 ₁₅₉₋₁₆₇ tetramer ⁺ CD8 ⁺ cells (%)
PBC 2	0.52	CLD*1 1	< 0.1
PBC 3	1.13	CLD 2	< 0.1
PBC 5	0.53	CLD 3	< 0.1
PBC 8	0.13	CLD 4	< 0.1
PBC 9	0.14	Normal 1	< 0.1
PBC 10	0.24	Normal 2	< 0.1
PBC 13	0.82	Normal 3	< 0.1
PBC 14	0.36	Normal 4	< 0.1
PBC 15	0.66	A2 neg PBC*2 25	< 0.1
PBC 16	0.27	A2 neg PBC 26	< 0.1
PBC 17	0.12	A2 neg PBC 27	< 0.1
PBC 18	1.20	A2 neg PBC 28	< 0.1
Mean ± SD	0.42 ± 0.33*3		

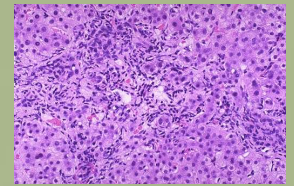
*1: CLD = chronic liver diseases controls

*2: A2 neg PBC = HLA A2 negative PBC

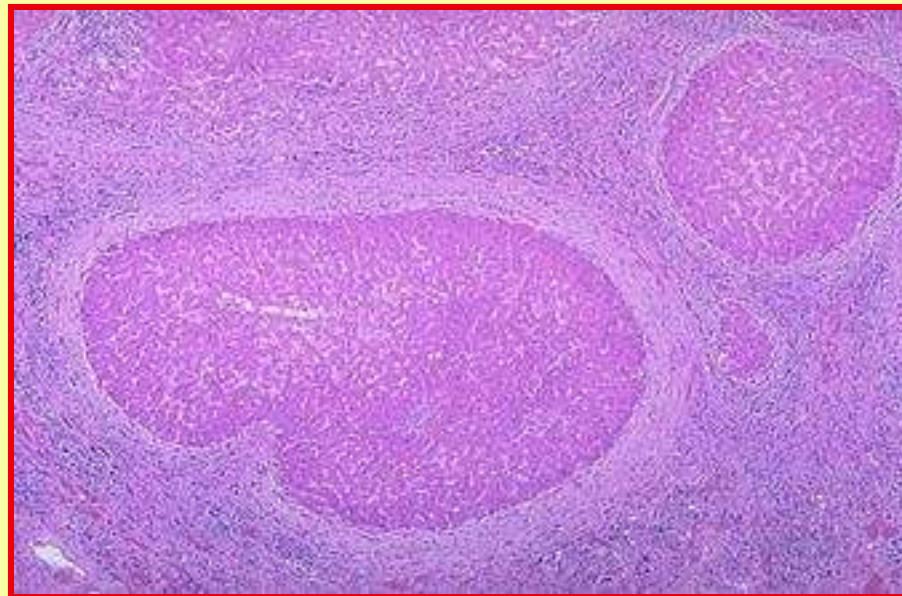
*3: Values for PBC patients were significantly (P<0.0001) higher than those from controls (CLD, Normals, and A2 neg PBC).

PATHOLOGIC FEATURES

Inconvenient Truths



In early histologic lesions there is eosinophilia, and also granulomas that are unique to PBC versus other liver



disease except for sarcoidosis , and are unique versus autoimmune pathologies overall. Granulomas have led to suspicions of an microbial basis for PBC , but this has not been established, nor can retroviral infection be substantiated .

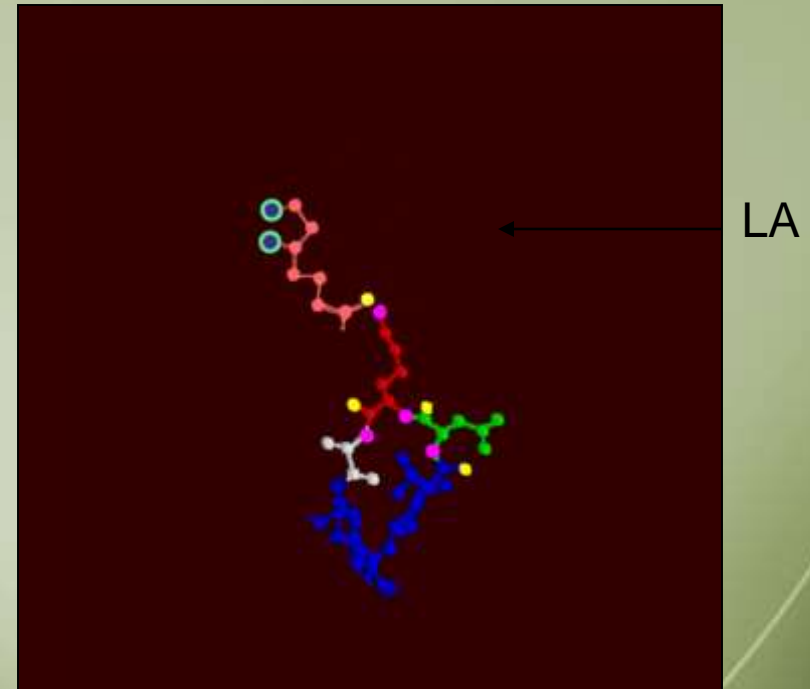
Immunologic features:

Convenient truths.

2-oxo-acid dehydrogenase complexes (2-OADC)

The autoantigens include the E2 subunits of the pyruvate dehydrogenase complex (PDC-E2), the branched chain 2-oxo-acid dehydrogenase complex (BCOADC-E2), the 2-oxo-glutaric acid dehydrogenase complex (OGDC-E2), and additionally the dihydrolipoamide dehydrogenase binding protein (E3BP) . All of these protein have at least one lipoyl domain (lipoic acid) (LA).

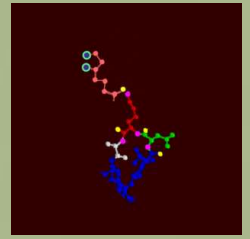
LA is exposed on PDC-E2, swings about, and is a ideal target.



Highly homologous proteins are found throughout the bacterial world.

Immunologic features:

Convenient truths.



Overlapping epitopes

Human PDC-E2

B cell ***KVGEKLSEGDLLAEIETDKATIGFEVQEEGY***

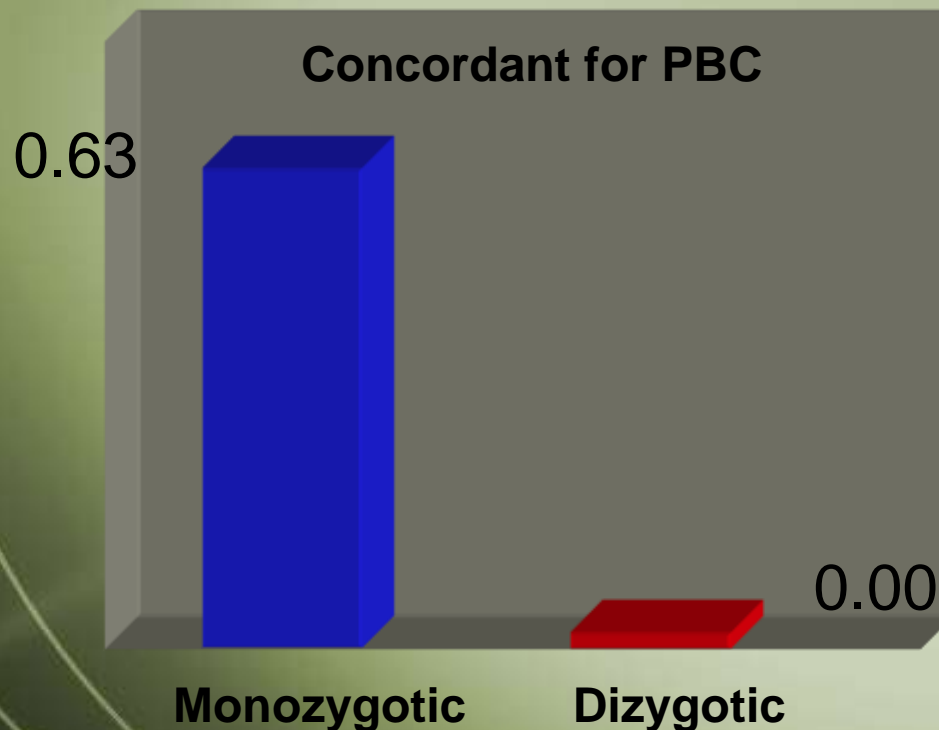
CD4 KVGEKLSE ***GDLLAEIETDKATIGFEVQEEGY***

CD8 KVGE ***KLSEGDLLAEIETDKATIGFEVQEEGY***

Assays using these epitopes have found **100-150 fold** increase in the number of autoreactive CD4+ T cells and a **10 fold** increase in the number of autoreactive CD8+ T cell found in the liver compared to the peripheral blood.

GENETIC INFLUENCES

Convenient truths.



In **5 of 8 sets of the monozygotic twins**, both individuals had PBC (0.63 concordance) but among the **8 dizygotic twin** pairs, **none** were concordant for PBC. Interestingly, the age at onset of disease was similar in 4 of the 5 concordant monozygotic twin pairs. Hence, while the concordance rate of PBC in identical twins is among the highest reported for any autoimmune disease, some discordant pairs were identified.



ENVIRONMENTAL INFLUENCES

Convenient truths

From our epidemiology study we found an association with;

History of urinary tract infections,
Past cigarette smoking,
Use of reproductive hormone replacement
Frequent use of nail polish.

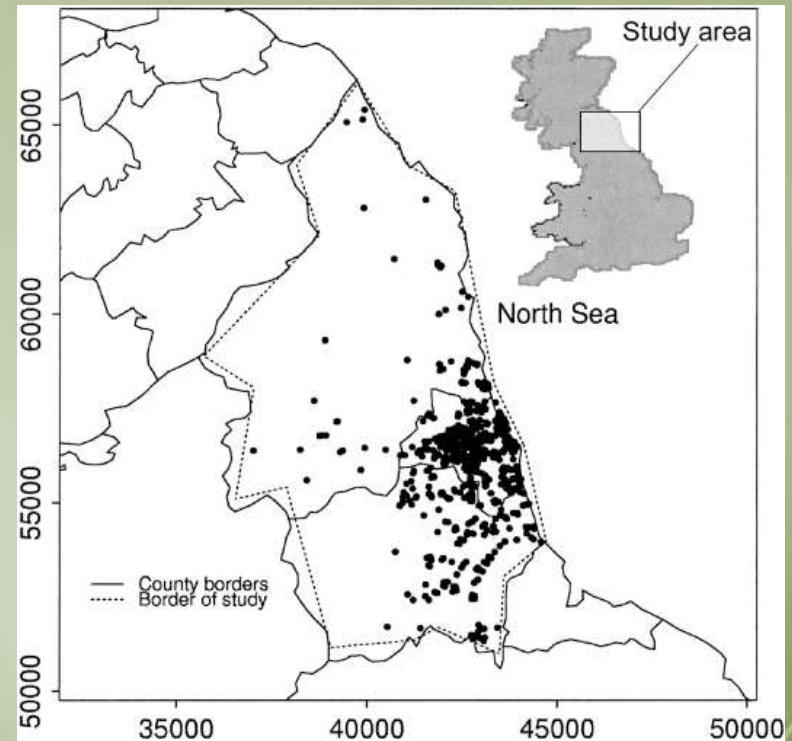
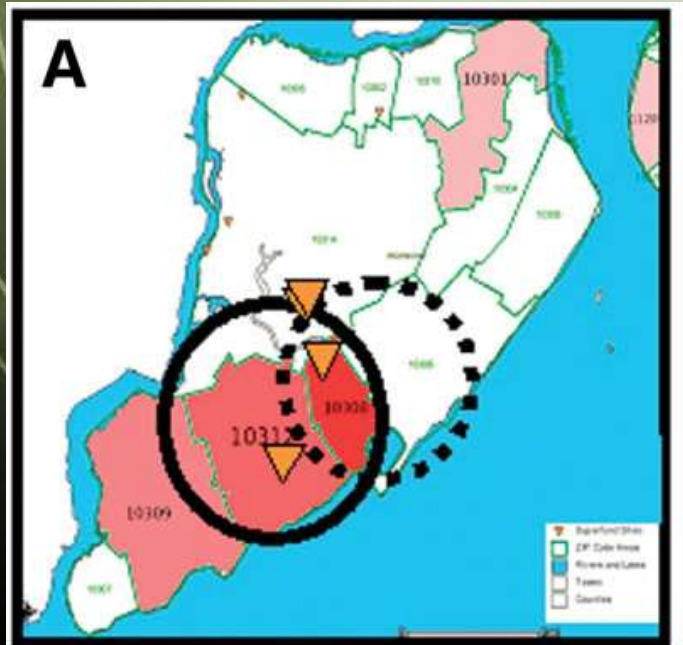


ENVIRONMENTAL INFLUENCES



Convenient truths

Toxic Superfund site
clustering of PBC in New
York



Clustering of PBC
cases in Northern UK

ENVIRONMENTAL INFLUENCES

Convenient truths



Million Tons of man made chemicals



~100,000 existing chemicals

Schematic of Peptide-Xenobiotic Microarray

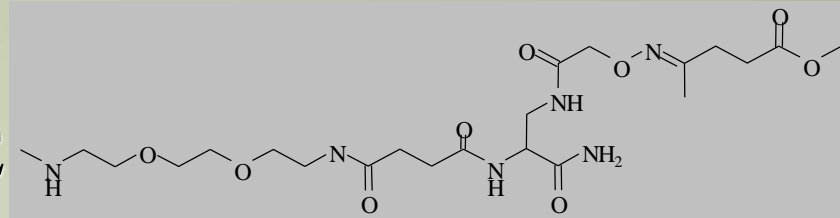
PDC-E2 peptide



X

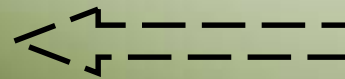
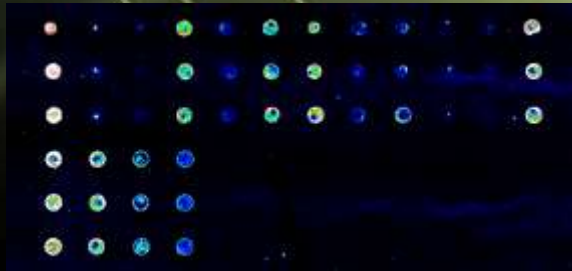
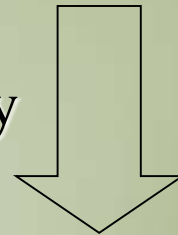
X: xenobiotic mimeotope

linker

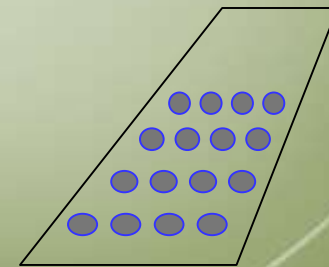


agarose

Spot onto microarray
glass slides

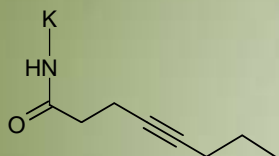


- 1) Block
- 2) Serum
- 3) Detection Ab (cy3/5-anti-IgG/M)
- 4) Dry & scan

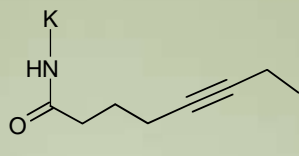


Name and Structure of Alkynoic Compounds

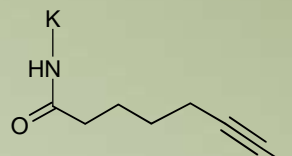
Alternating triple bond position



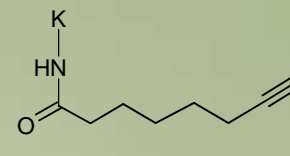
4-octynoic acid



5-octynoic acid

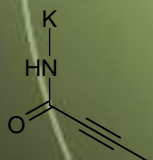


6-octynoic acid

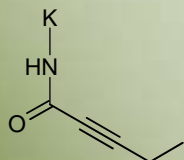


7-octynoic acid

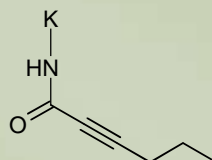
Alternating chain length



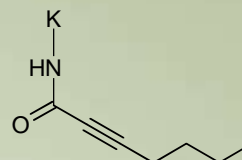
2-butyneic acid



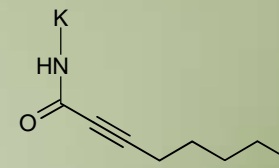
2-pentyneic acid



2-hexyneic acid



2-heptyneic acid



2-octyneic acid



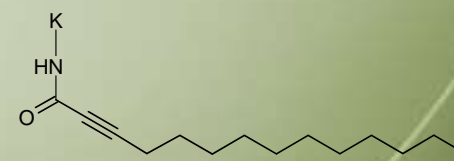
2-nonyneic acid



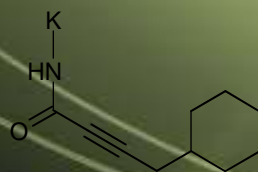
2-decyneic acid



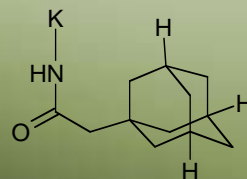
2-dodecyneic acid



2-tetradecyneic acid



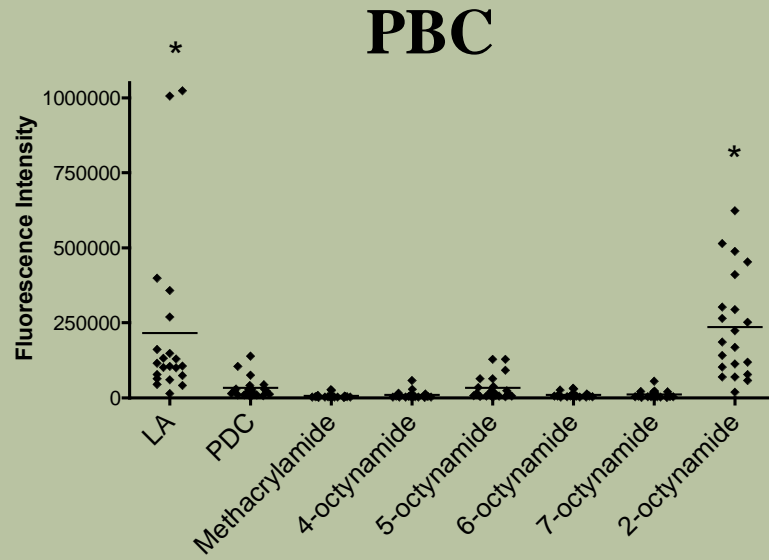
2-cyclohexyl-
4-butyneic acid



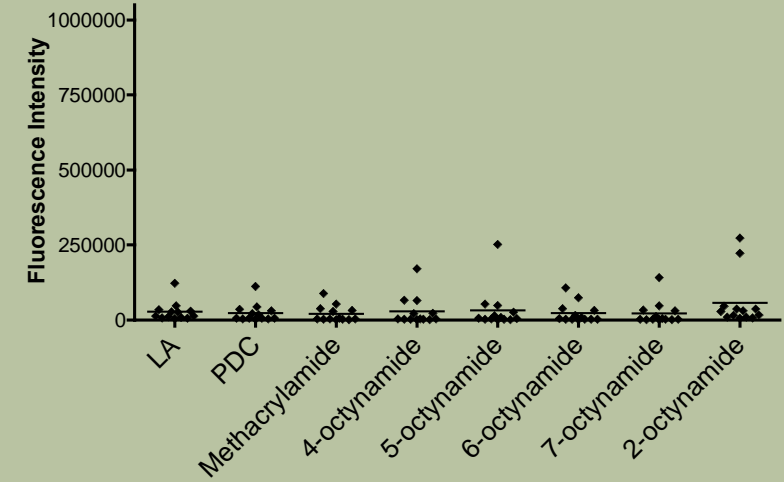
1-adamantyl-acetic
acid

Reactivity of n-octynamides with PBC sera

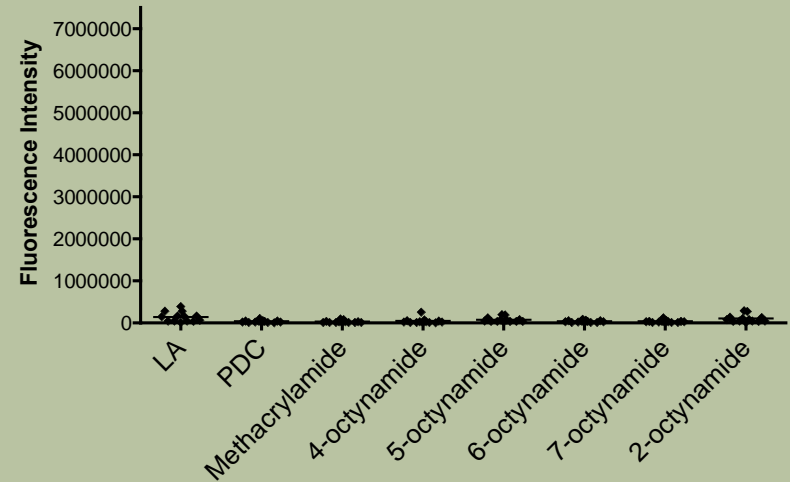
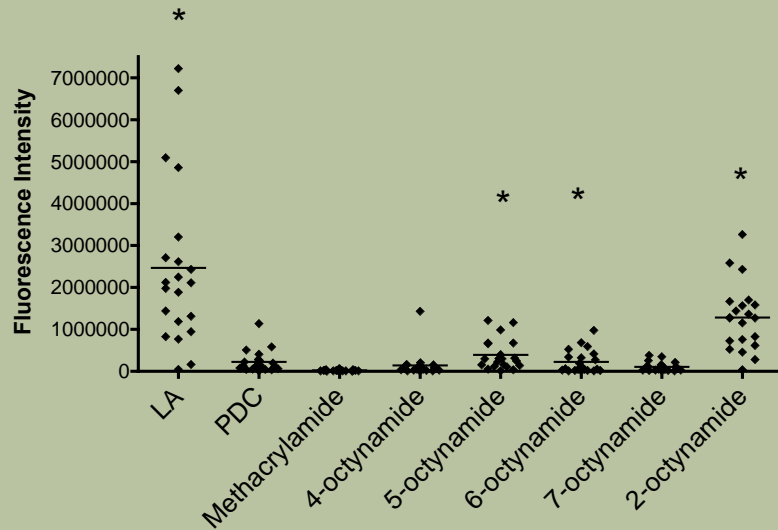
IgG



Control

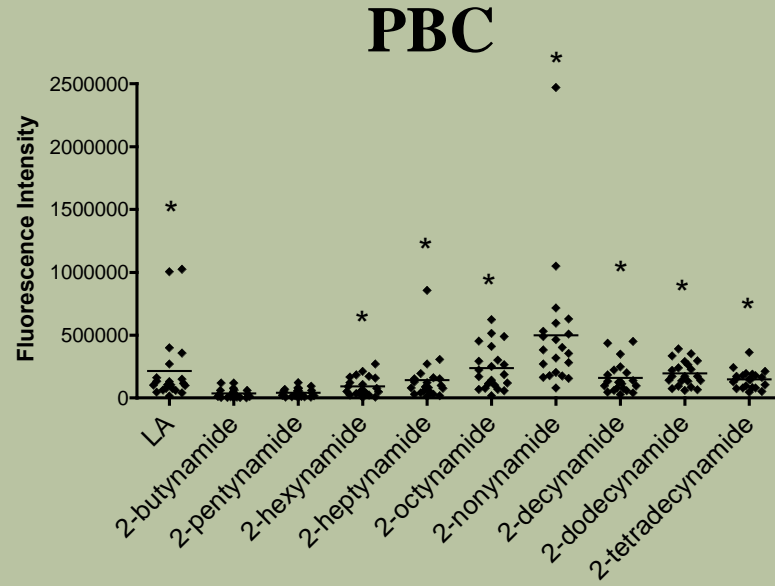


IgM

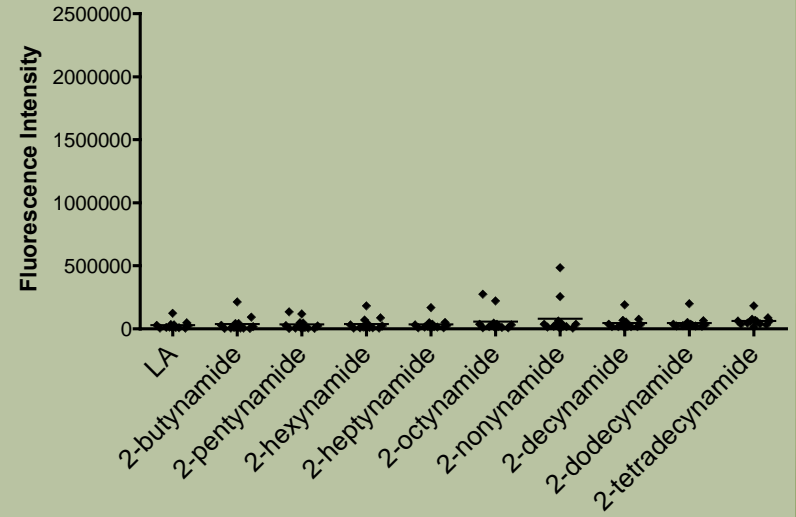


Reactivity of 2-alkynamides with PBC sera

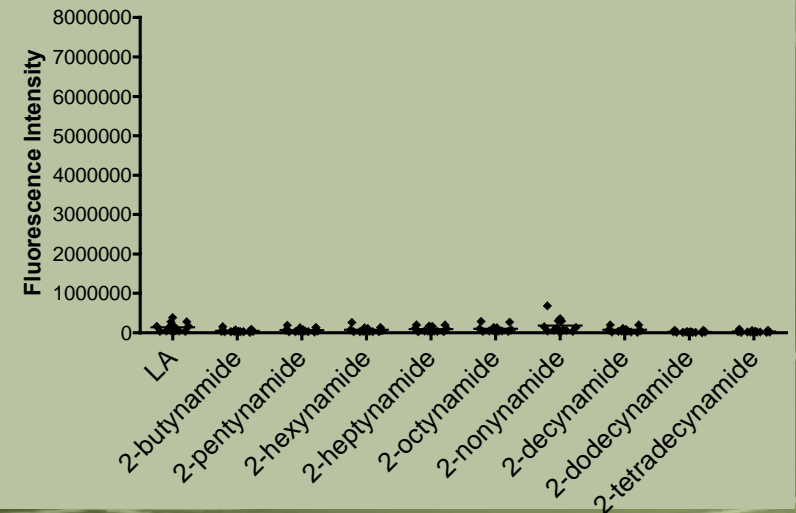
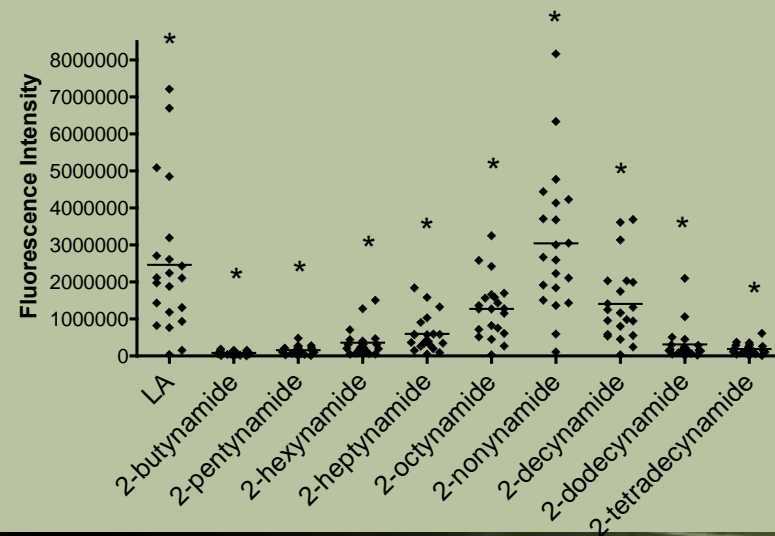
IgG



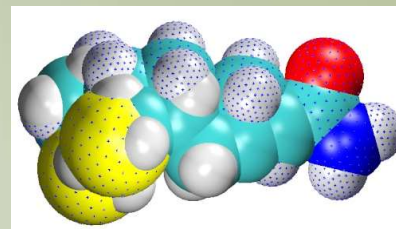
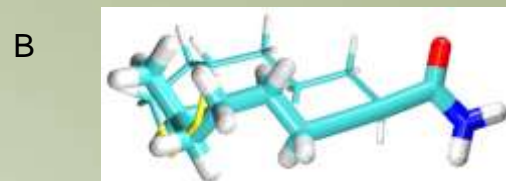
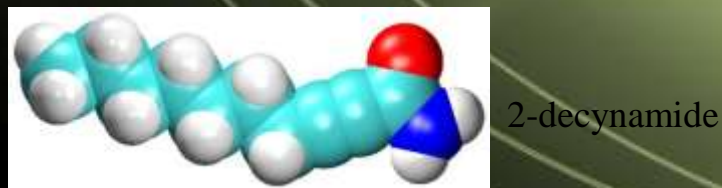
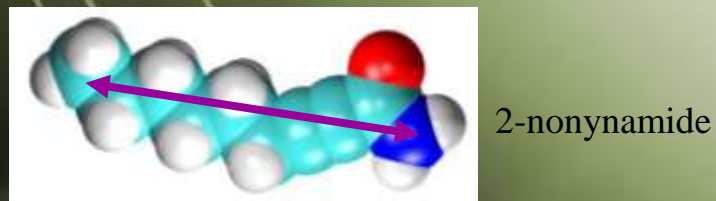
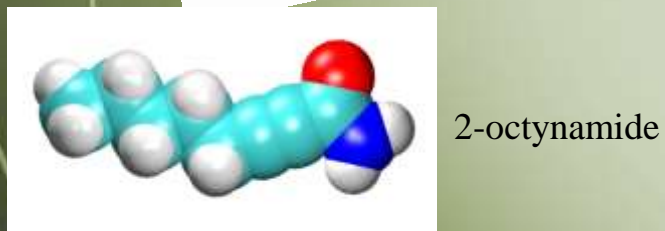
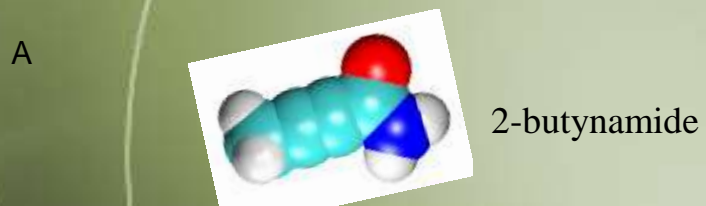
Control



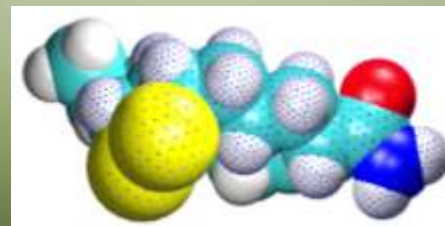
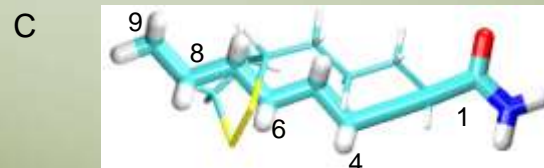
IgM



Molecular mimicry between lipoamide and 2-nonynamide

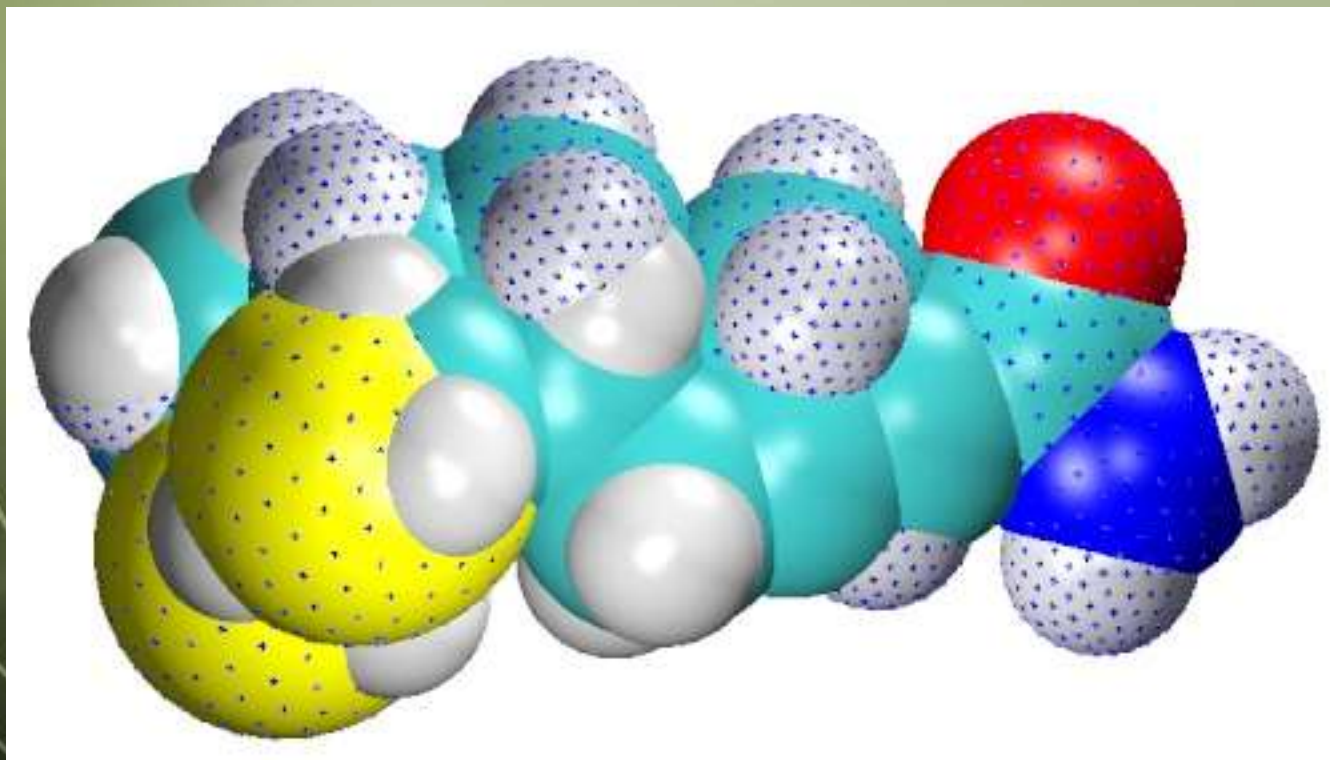


lipoamide vs.
2-nonynamide,
corkscrew
conformation



lipoamide vs.
2-nonynamide,
extended
conformation

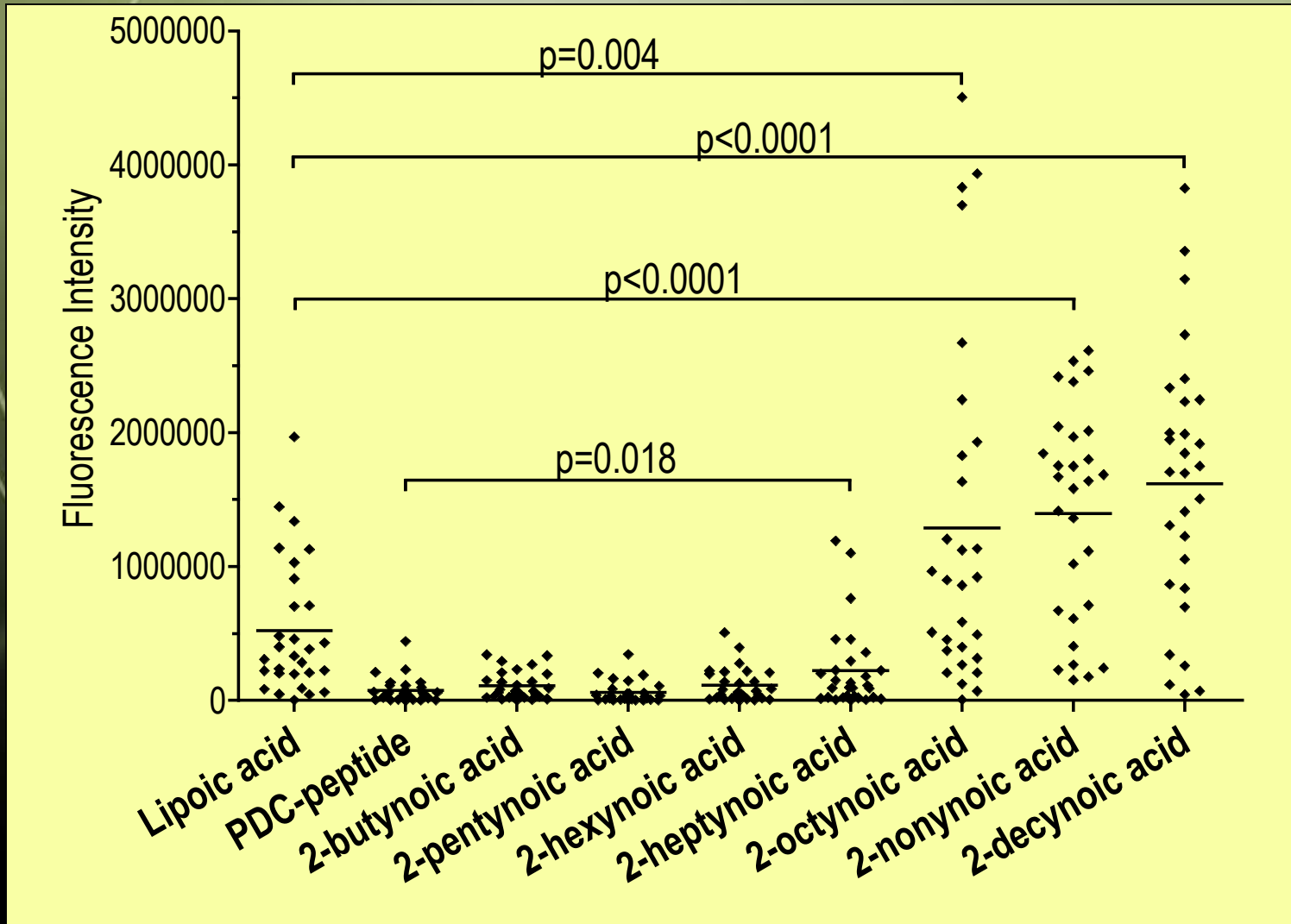
Molecular mimicry between lipoamide and 2-nonynamide



Superimposed models of lipoamide (dotted) vs. 2-nonynamide in corkscrew conformation

ENVIRONMENTAL INFLUENCES

Convenient truths



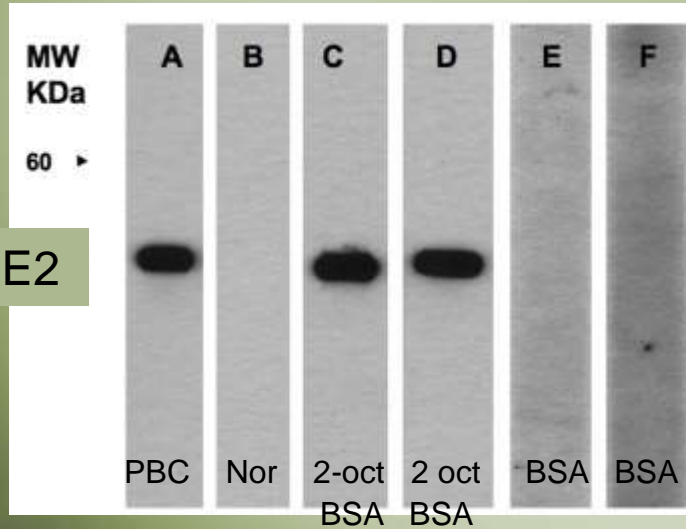
ENVIRONMENTAL INFLUENCES



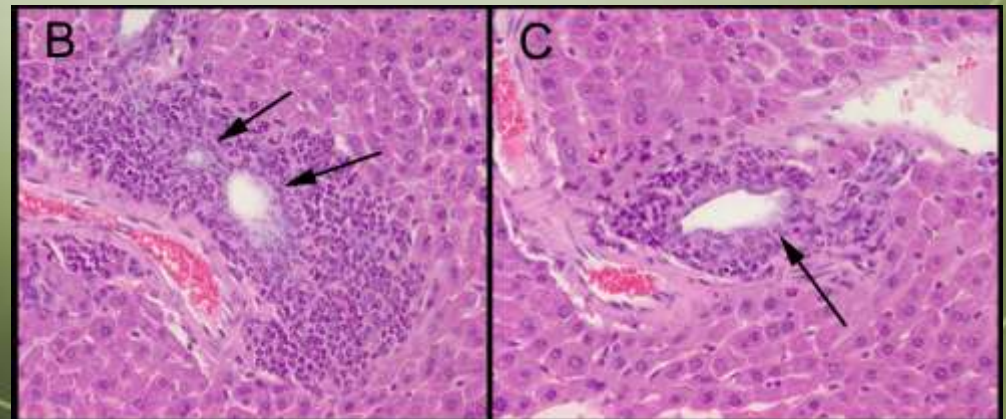
Convenient truths

Xenobiotics in mice

rPDC-E2



AMA of 2-octonyoic -
BSA immunized mice



Yuki Morotoki and the Mouse Group Family at UC Davis-Gershwin Lab



dnTGFβRII

- **Lack or aberrant activity of TGF-β signaling contributes to a loss of self tolerance to autoantigenic proteins in the liver, which in turn leads to autoimmunity and more toward activation of an intrinsically self-reactive T cell repertoire in which necessary regulatory T cell (T reg) influences are lacking**
- **dnTGFβRII mouse is an animal model in which immunoregulatory defects within the lymphocytic and phagocytic components of the immune system, potentially in association with a particular vulnerability of the biliary ductular target tissue, initiate an autoimmune response resembling that seen in PBC**

Characteristic of IL-2R α ^{-/-} mice

- 1. Increased lymphocyte number**
- 2. Massive enlargement of spleen and lymph node**
- 3. Autoimmune disorders, including hemolytic anemia and inflammatory bowel disease**
- 4. Body weight loss with diarrhea**
- 5. Premature death with severe anemia**





AMA Antibodies, Apoptose and APC



Blebs from Apoptotic Bile duct cells



Complex formation/Cytokine secretion



Anti-Mitochondrial Antibodies (AMA) ,Apotope and APC

~90% of Primary Biliary Cirrhosis patients have a significant AMA titer

AMA antibodies are rarely found in other diseases

AMA antibodies precedes liver PBC pathology

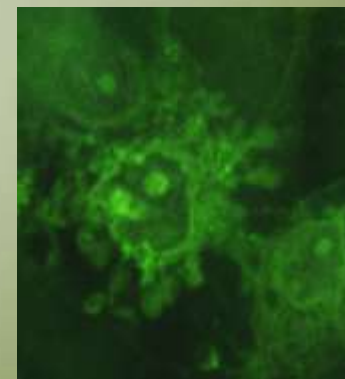
Role of AMA antibodies in the pathology of PBC has never been elucidated

Apotope on the surface of an early apoptotic cell interacts with the Ig receptor on a specific B cell

APC internalized the antigen from late apoptotic cells and lead the stimulation and generation of autoreactive T cell



Epiphenomena?? or something more significant





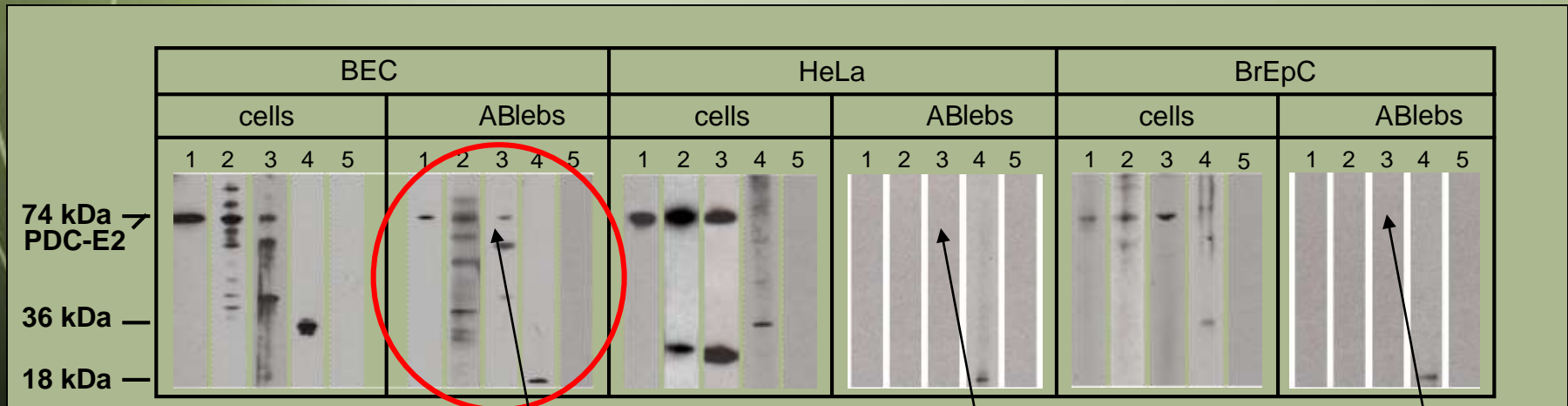
Complex formation--Blebs and Antibody and Cytokine profile

Can this complex (Blebs and AMA) be one of the initiators of liver inflammation in PBC and by what mechanism?

How do the cytokines play a role?

Background

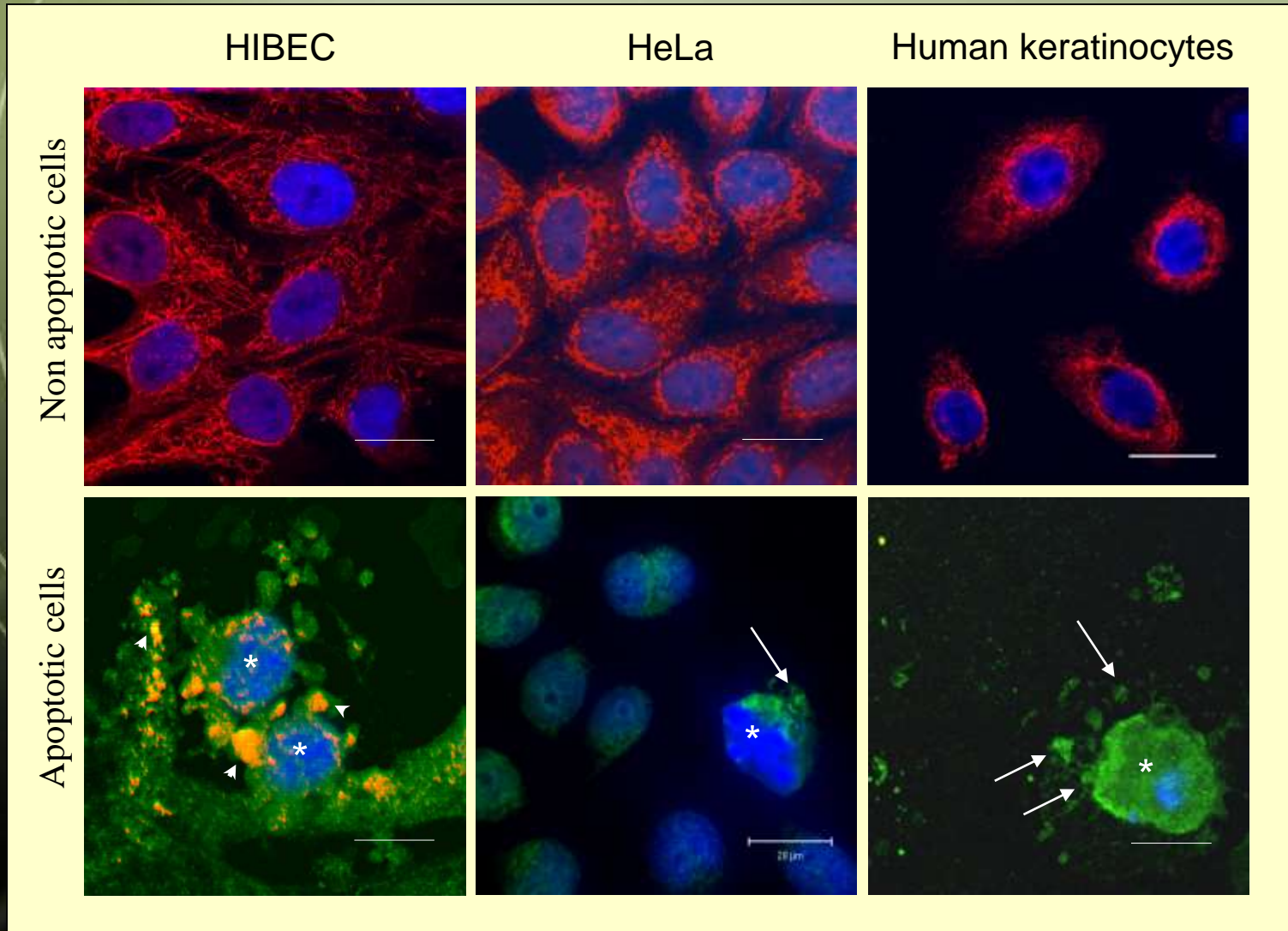
- PDC-E2 is left antigenically intact in apoptotic HIBEC cells
- PDC-E2 localizes within blebs



HIBEC cells are non transformed Bile duct cells

Background

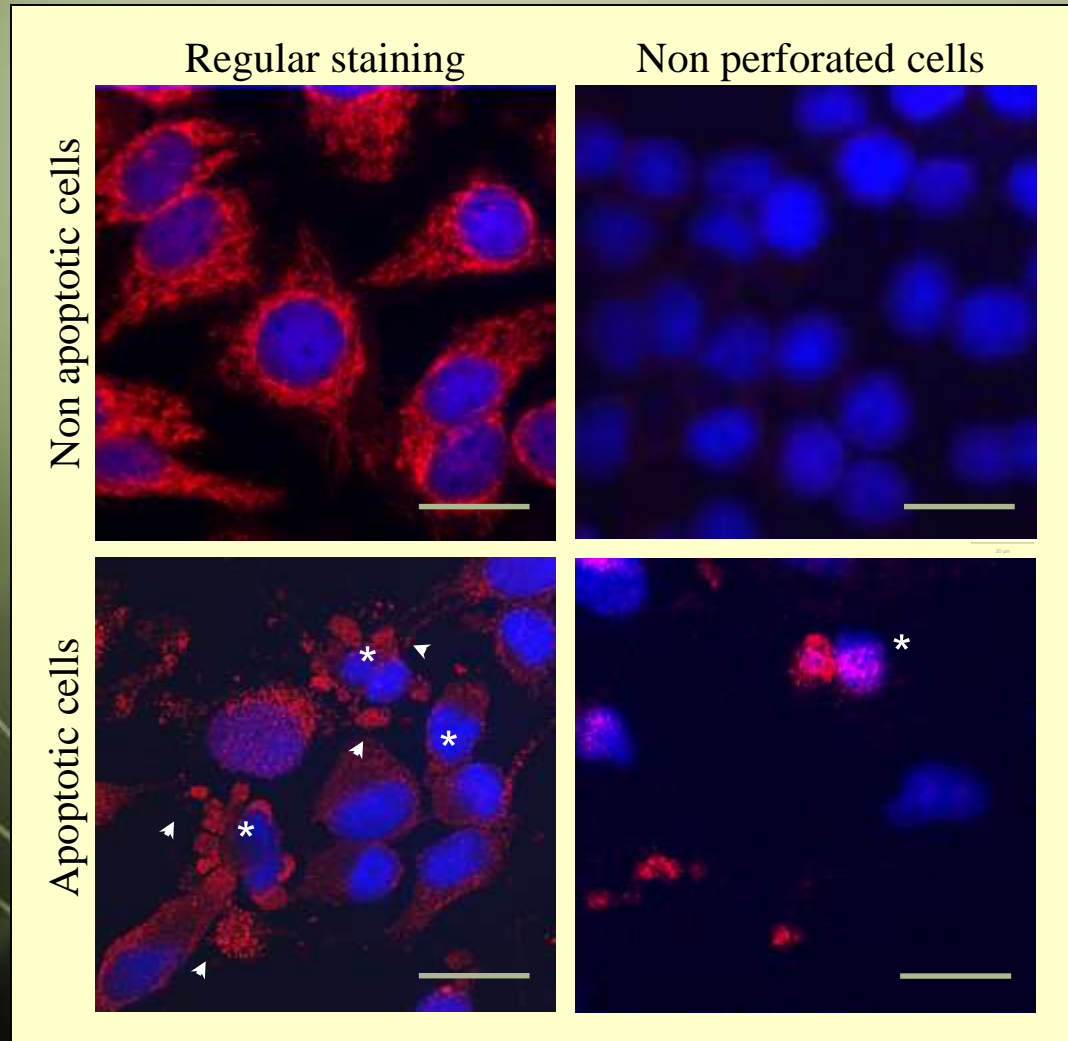
- Localization of PDC-E2 within apoptotic blebs is HIBEC specific



merge = co-localization of monoclonal anti-PDC-E2 antibody and annexin 5

Background

- PDC-E2 in apoptotic blebs is accessible to antibody recognition



Red= anti-PDC-E2 antibody from PBC patient, Blue =DAPI nuclear stain. * = apoptotic cell

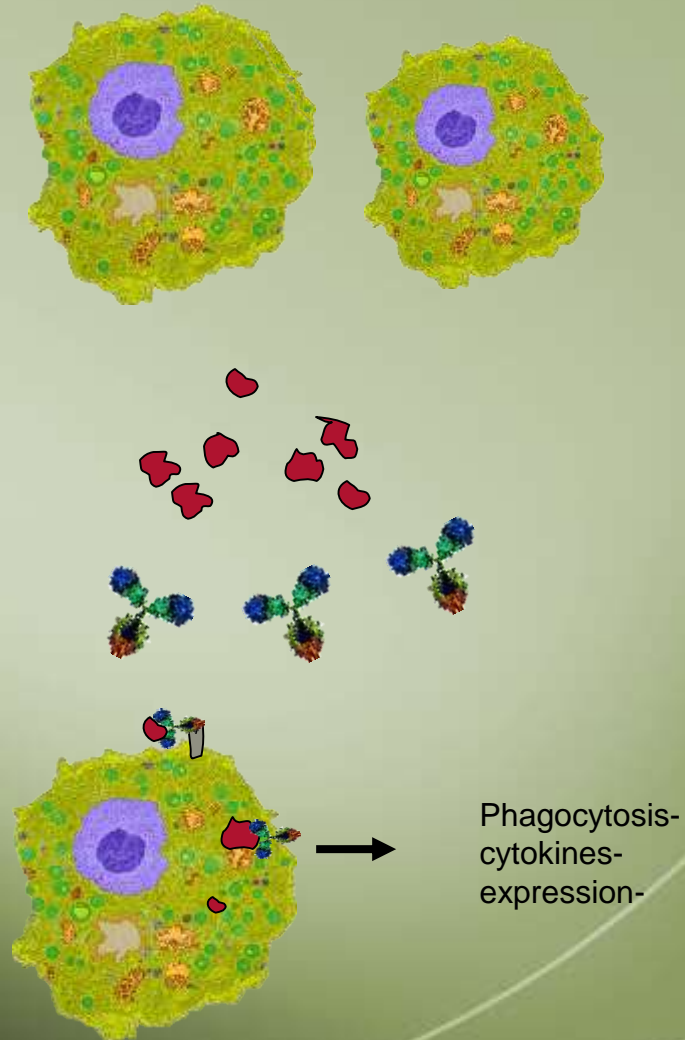
Design

1) Co-culture monocyte derived macrophages from PBC patients and controls

2) With purified HIBEC and control cell apoptotic blebs

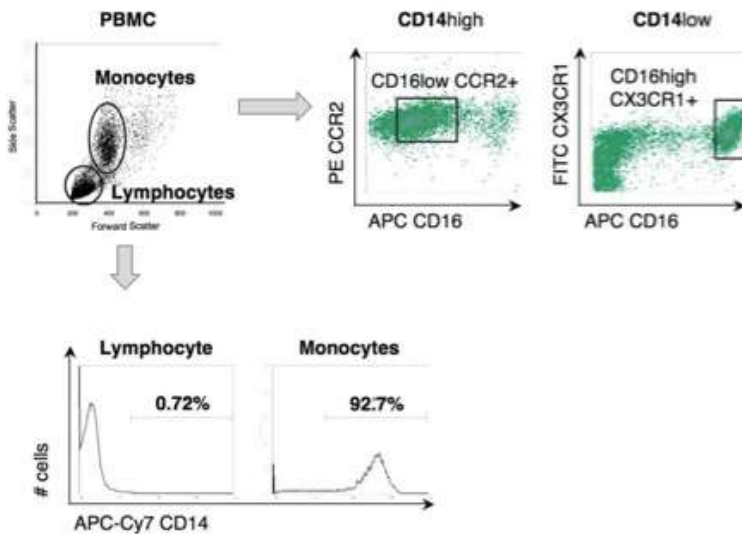
3) And with AMA IgG and control IgG

4) Characterize resulting immune parameters

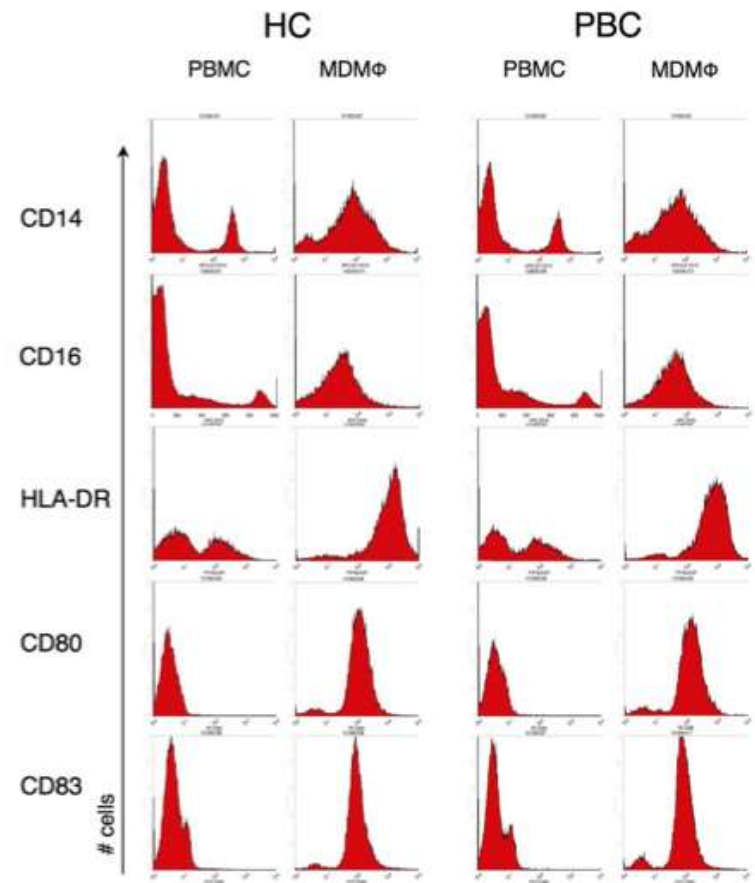


Monocyte derived macrophages from healthy controls (HC) and from PBC patients shows similar phenotypes

A



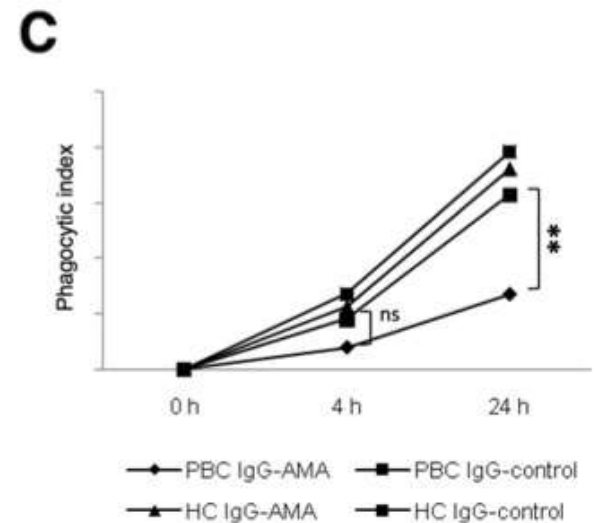
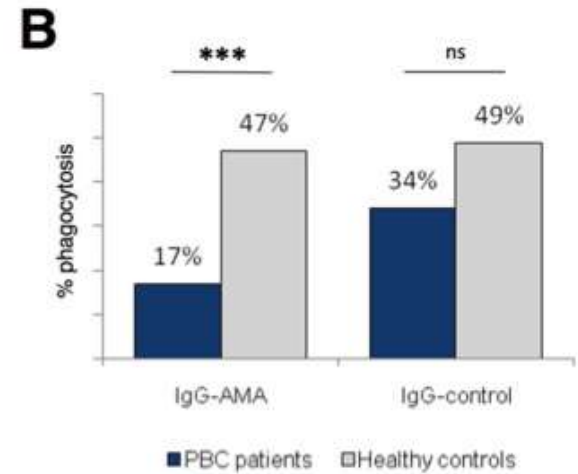
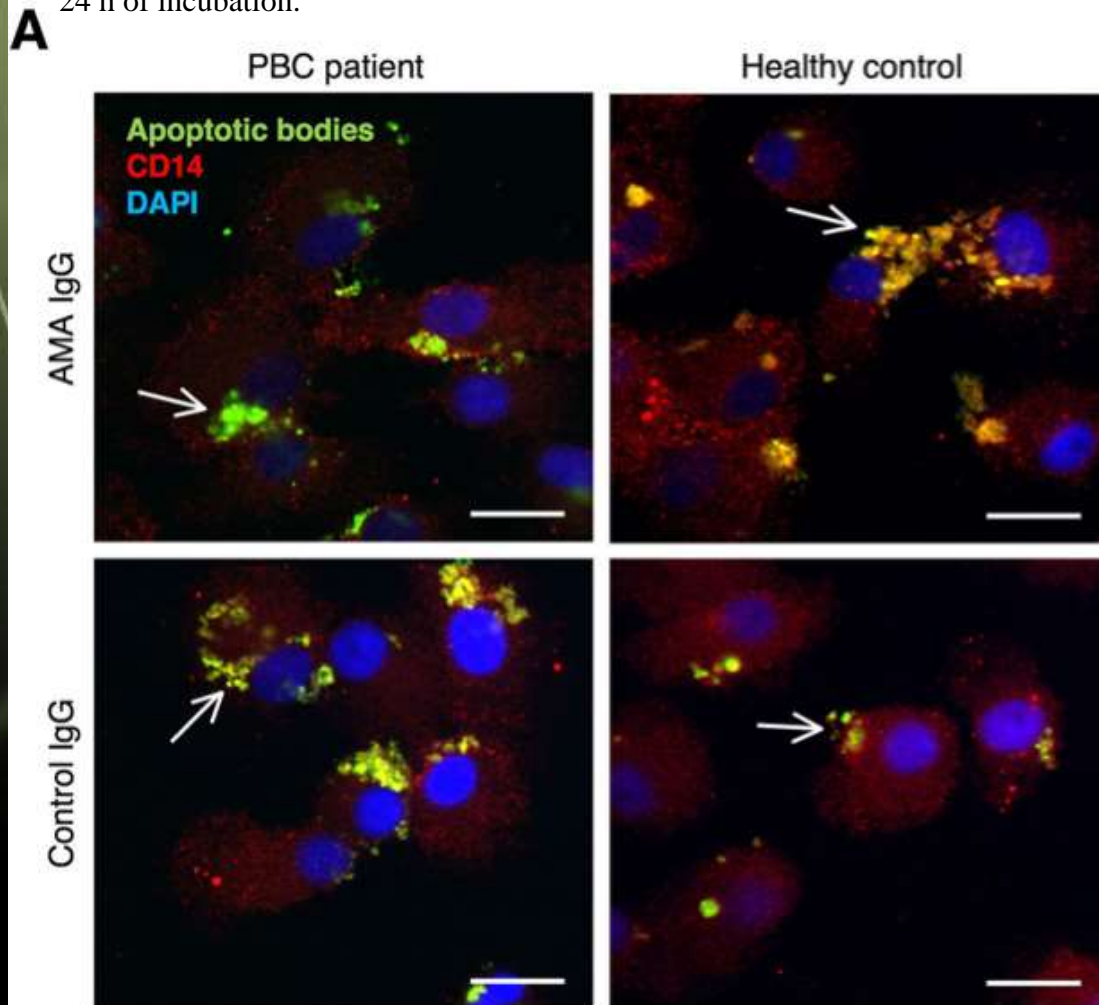
B



Uptake of apoptotic bodies. (A) Confocal imaging of MDM Φ . In all four conditions, green apoptotic bodies are located inside the cells which indicate that MDM Φ actively engulfed apoptotic bodies after 24 h (arrows). One representative section of 6 different subjects studied is shown for each condition; scale bar represents 20 μ m.

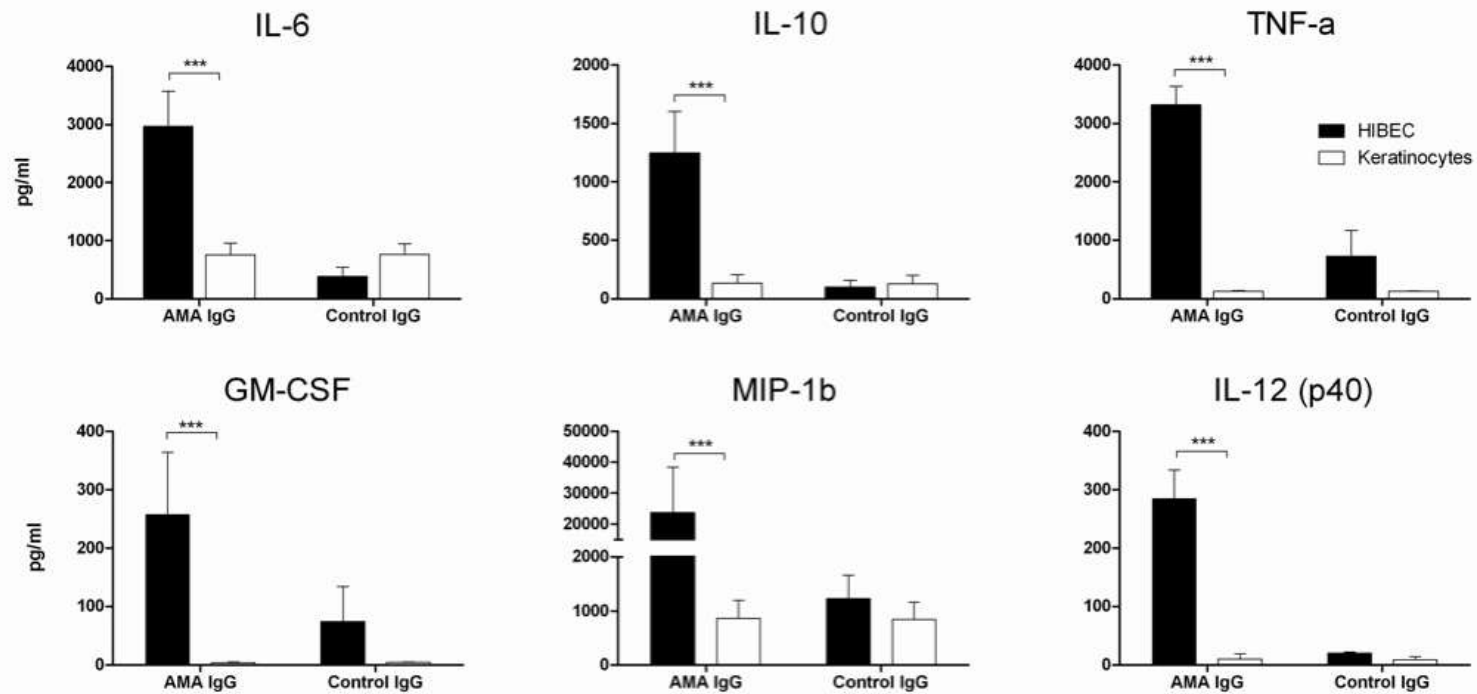
(B) Percentage of phagocytosis was calculated by counting the number of macrophages that had ingested at least one HIBEC apoptotic body. MDM Φ from patients with PBC had a reduced uptake of HIBEC apoptotic bodies in the presence of AMA compared to healthy controls (17% vs 47%, *** $p < 0.001$), this difference was not noted when IgG control was used (34% vs 49%, $p = ns$). (Fisher's Exact Test).

(C) The phagocytic index (PI) was expressed as percentage of phagocytosis multiplied by the mean number of phagocytosed bodies per macrophage (ABM Φ): $PI = (\% \text{ phagocytosis} \times \text{mean ABM}\Phi / 100)$ and was evaluated at 0, 4 and 24 h of incubation.



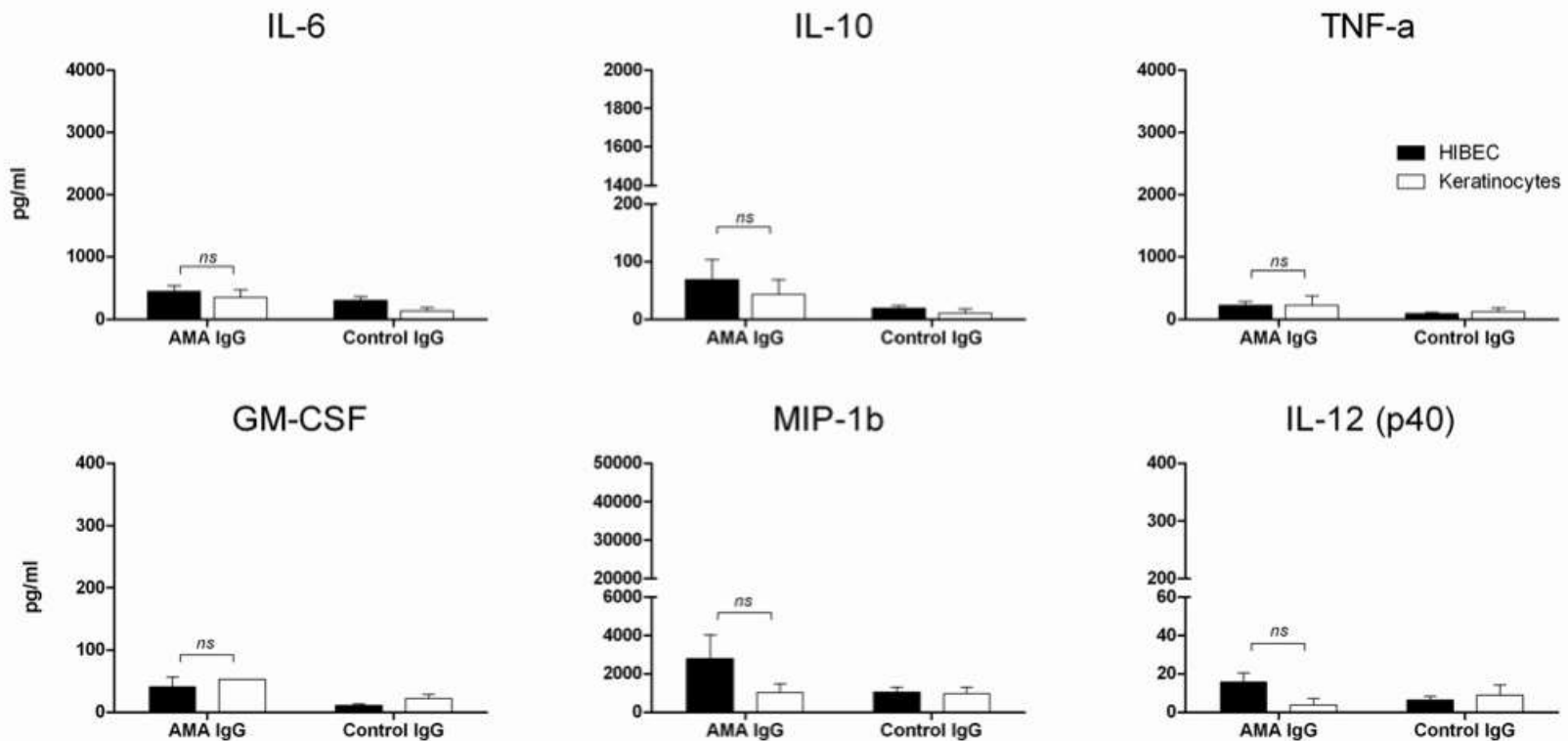
PBC macrophages cocultured with HIBEC and keratinocyte blebs and AMA IgG or Control IgG

A. PBC

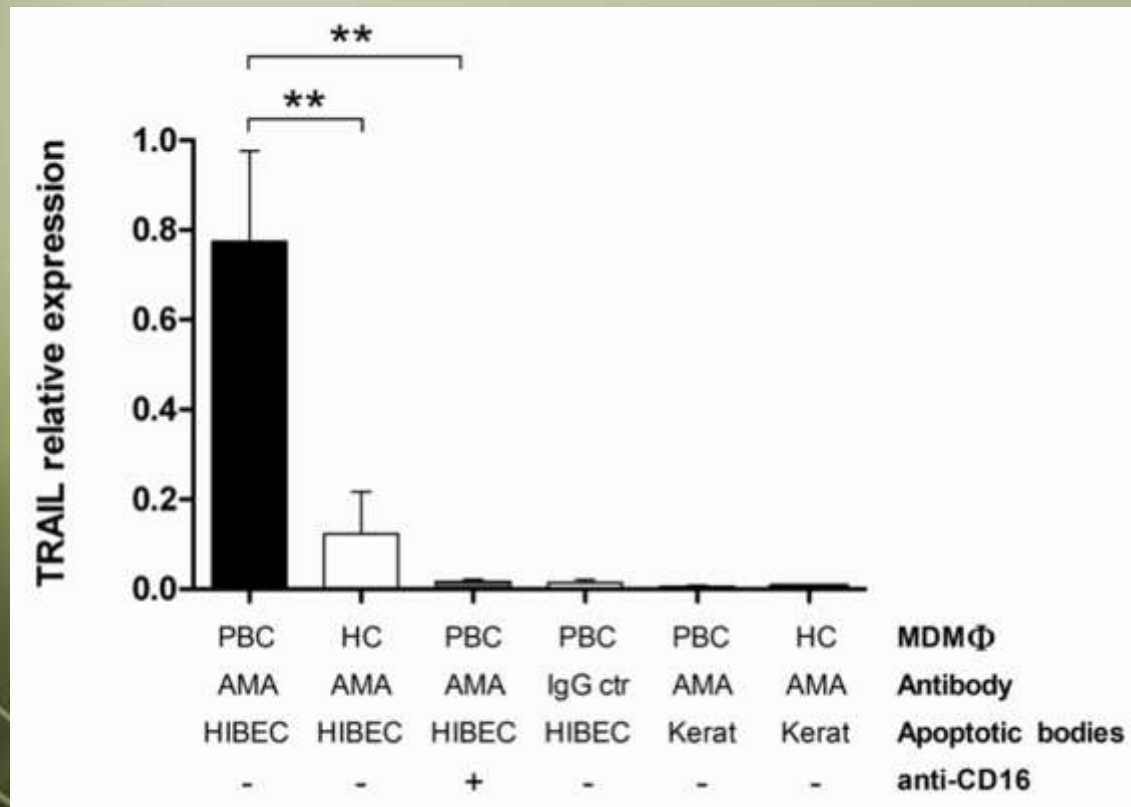


Healthy control macrophages cocultured with HIBEC and keratinocyte blebs and AMA IgG or Control IgG

B. Healthy Controls

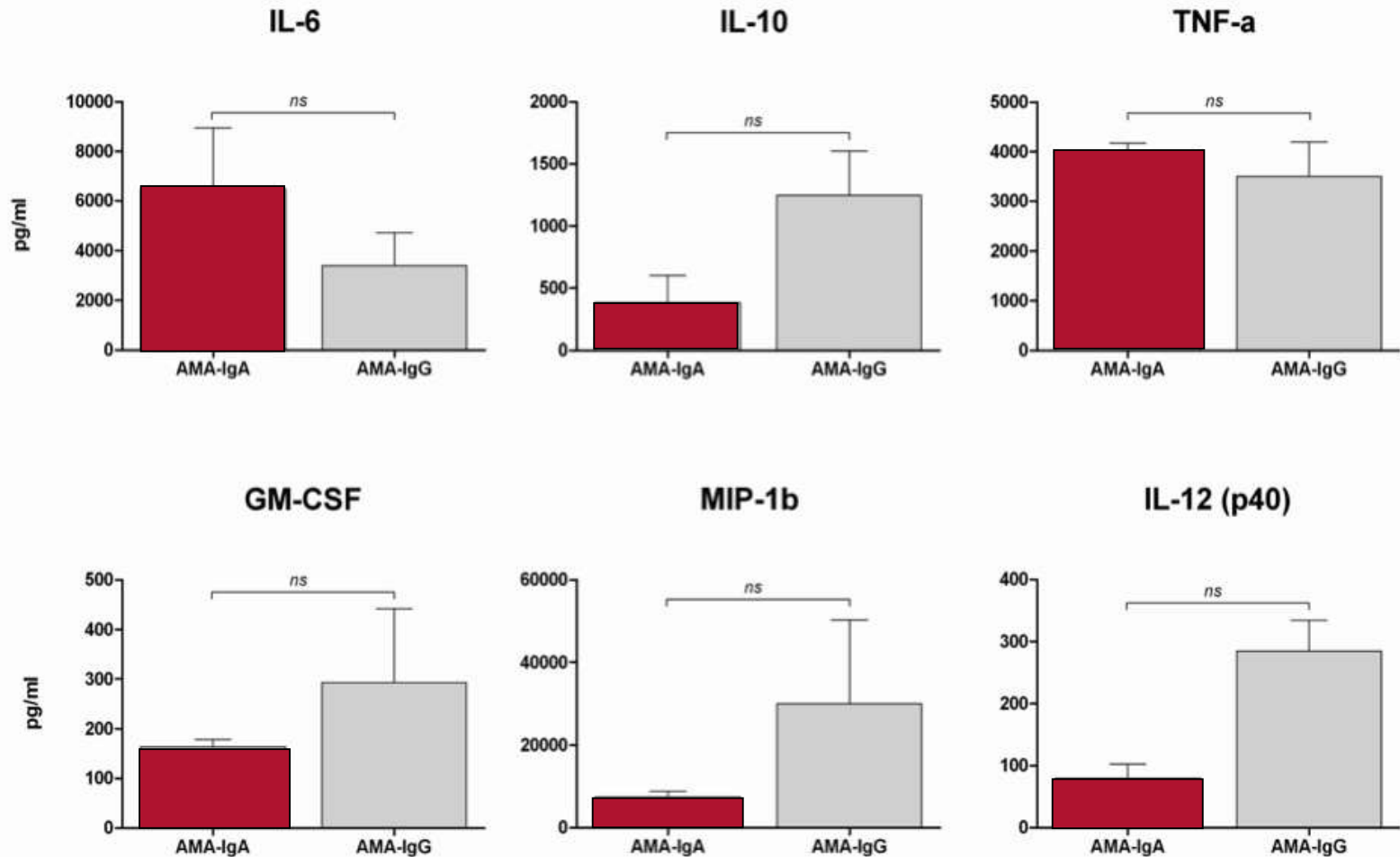


The expression of pro-apoptotic TRAIL is significantly increased in PBC macrophages cocultured with HIBEC and AMA IgG.

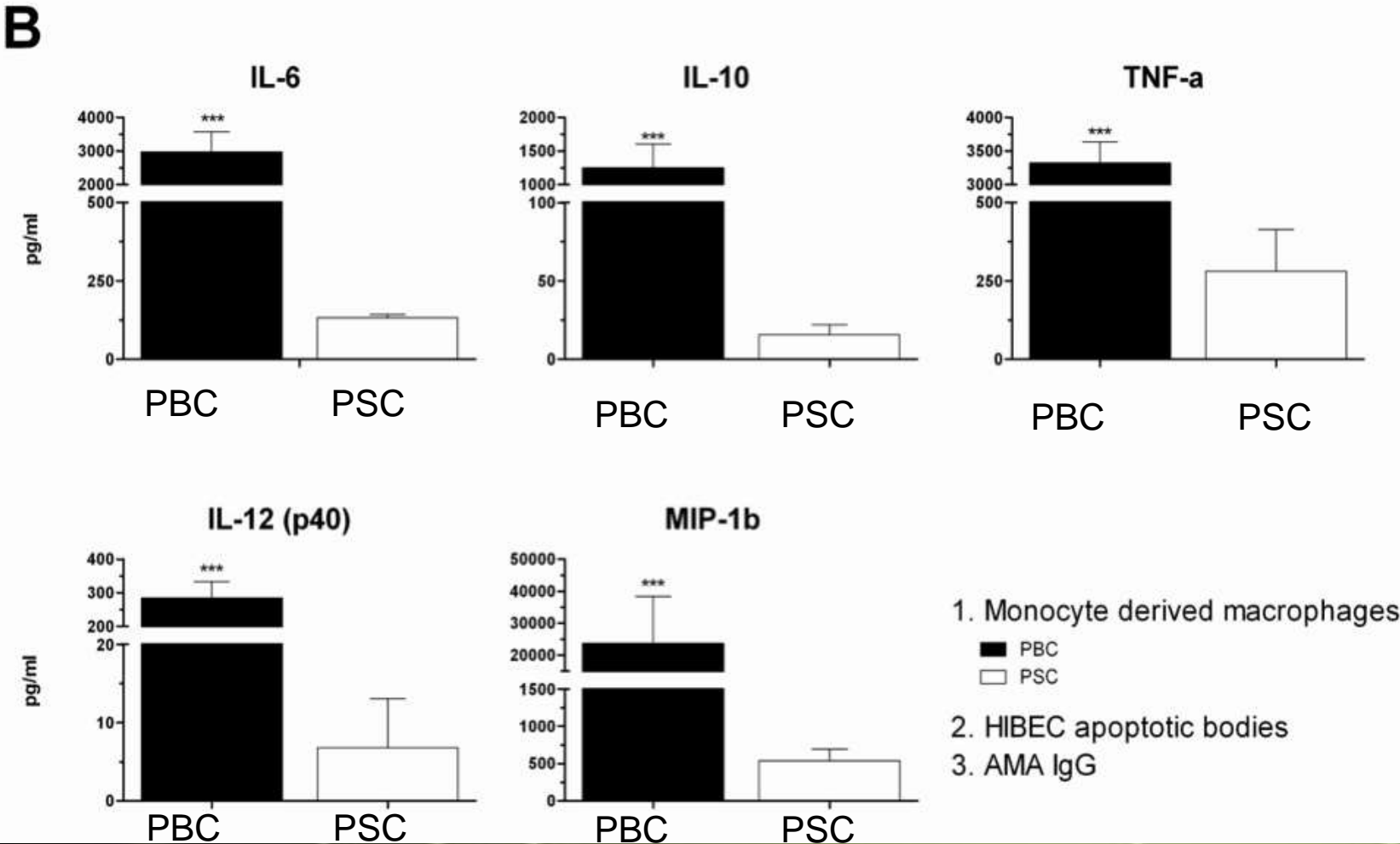


PBC macrophages cocultured with HIBEC blebs incubated and **AMA IgA** or AMA IgG

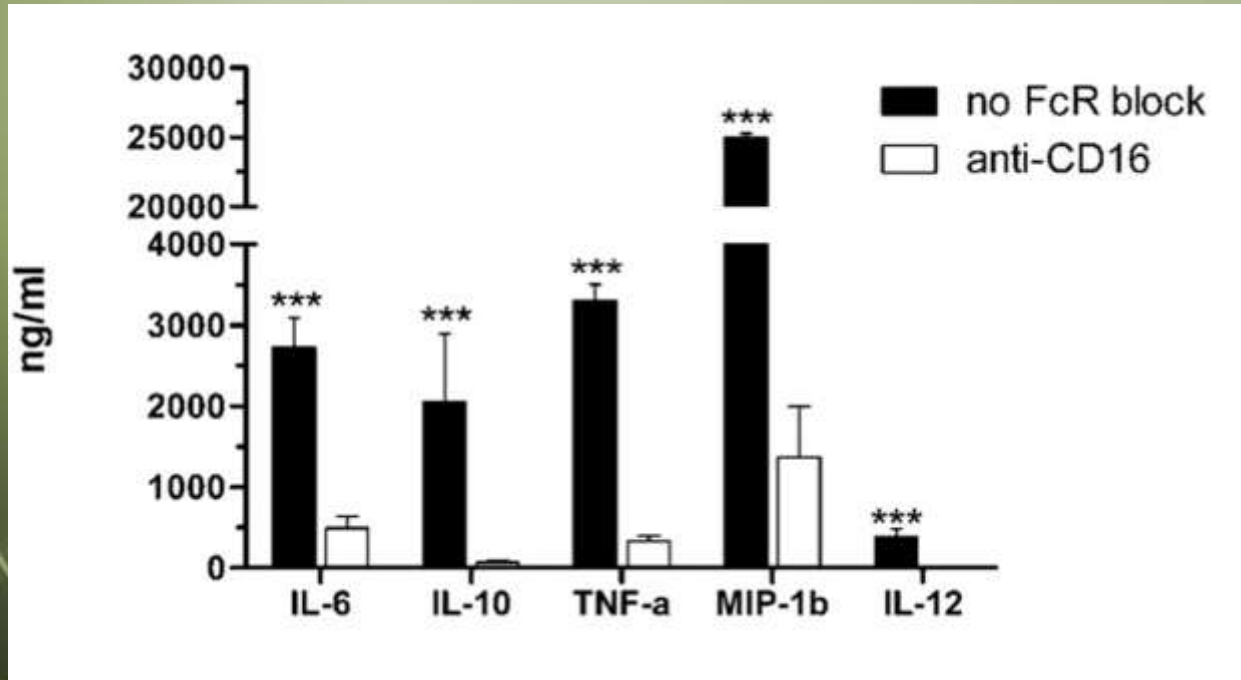
A



Macrophages from PBC and PSC patients with HIBEC blebs and incubated with AMA IgG



Blocking the Fc γ RIII (CD16) inhibits the cytokine release



Conclusions: our data indicate that the unique characteristics of BEC during apoptosis might constitute the pathogenic link between the ubiquitous nature and high degree of conservation across species of the AMA autoantigen and the organ specificity of PBC pathology.

Convenient truths

Currently, more than 20,000 people in the United States are waiting for liver transplants.

About 6000 liver transplantations were performed in the United States last year.

Convenient truths

Currently, more than 20,000 people in the United States are waiting for liver transplants.

About 6000 liver transplantations were performed in the United States last year.

Inconvenient truth.

About 2000 of these people will die waiting for a transplant.



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