AUTOANTIBODY PROFILING USING IMMUNOPROTEOMIC ANALYSIS OF PATIENT SERA WITH A NEW TYPE OF AUTOIMMUNE-LIKE HEPATITIS OCCURRING AFTER BONE MARROW TRANSPLANTATION

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❖ Liver dysfunctions after bone marrow transplantation (BMT) are multiple.

❖ The most common is the graft-versus-host disease (GVHD).

❖ The occurrence of autoimmune-like hepatitis developed *de novo* is exceptionally described in this situation.

AIM OF THE STUDY

- Report three cases of patients developing autoimmune-like hepatitis after bone marrow transplantation.

- Identify most of the autoantigens, using serological proteome analysis.

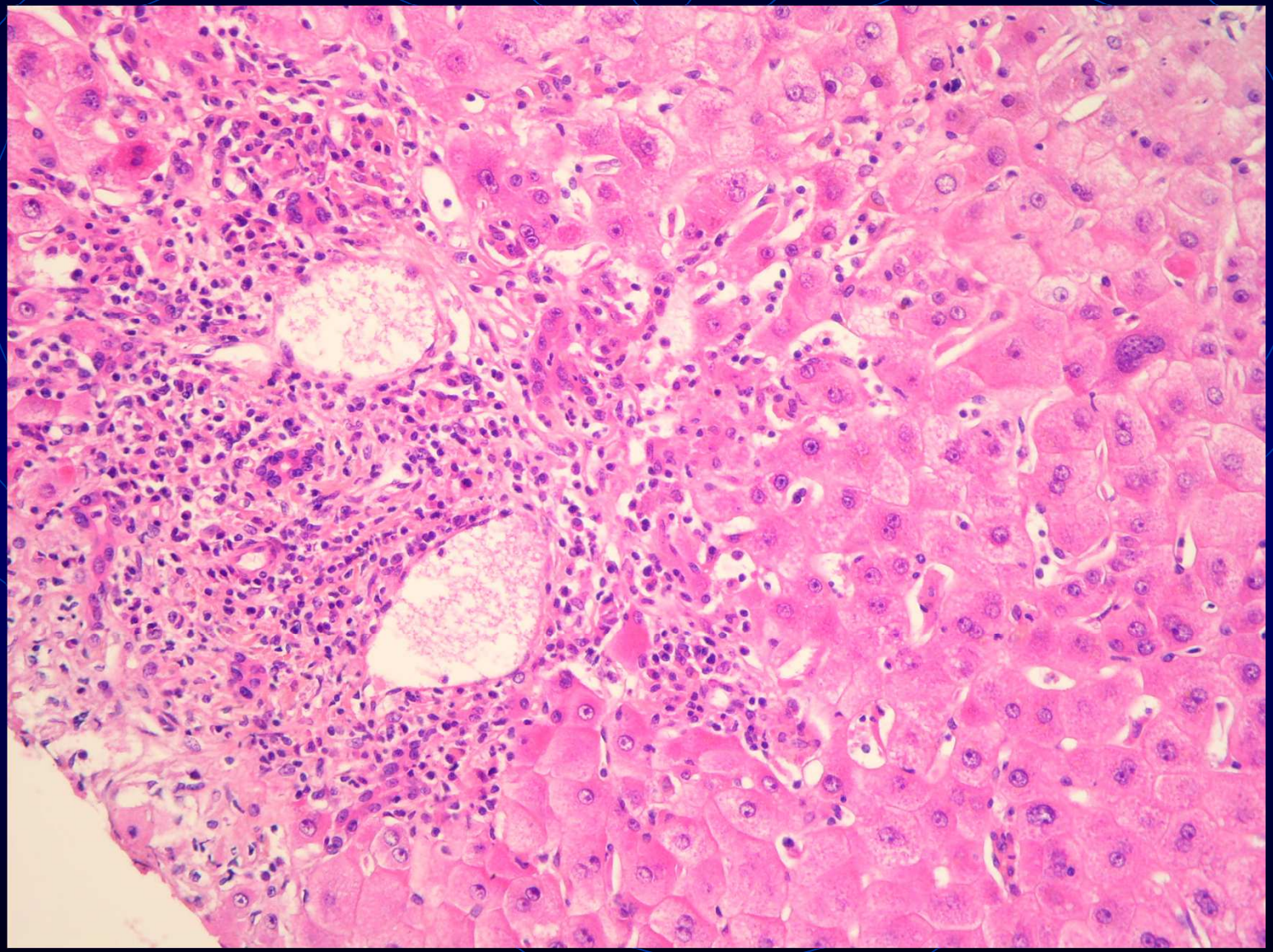
PATIENTS (1)

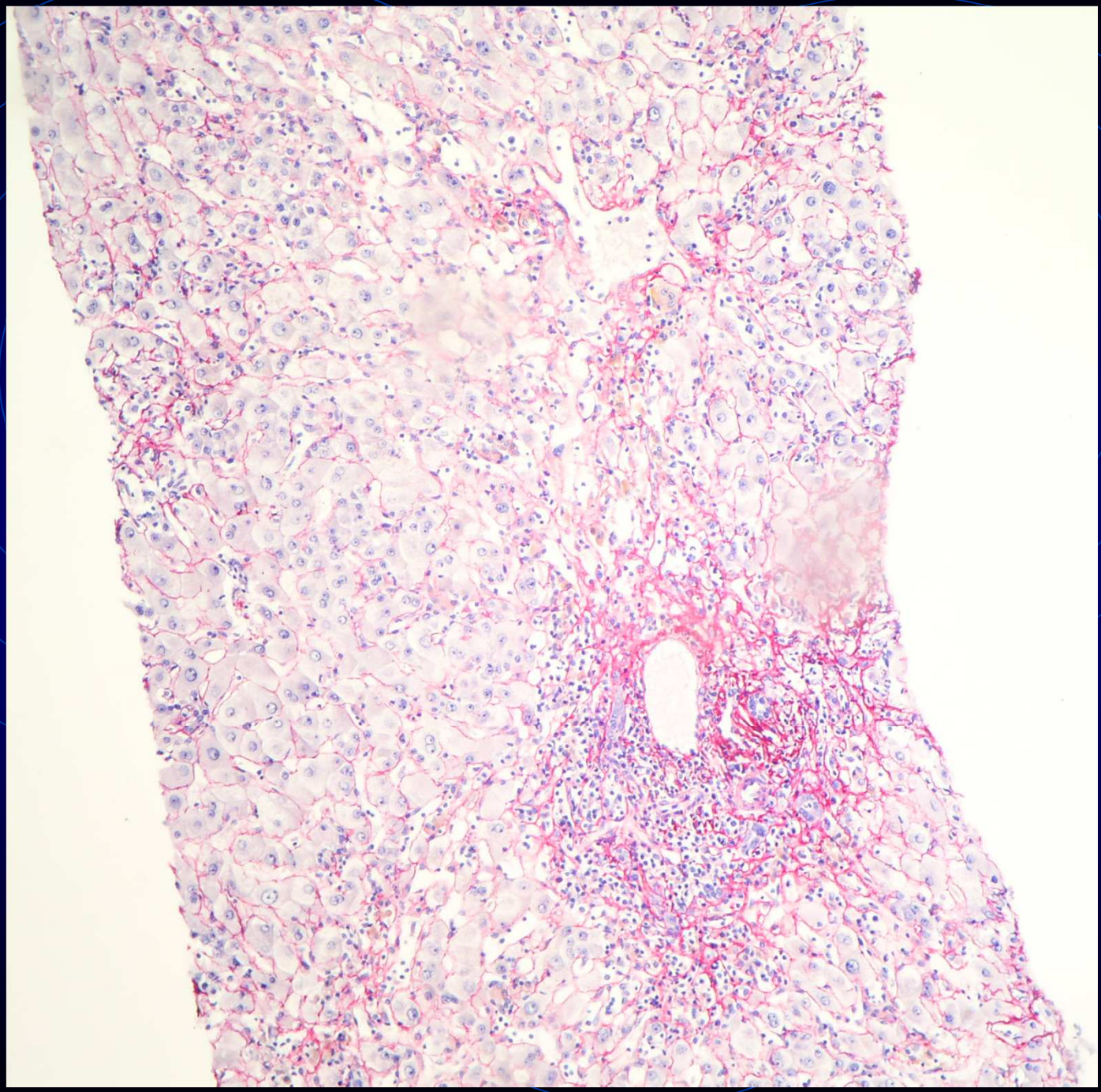
	Patient N°1	Patient N°2	Patient N°3
Hematologic disease	Myelodysplastic syndrome	Chronic lymphocytic leukaemia	Chronic lymphocytic leukaemia
Sex / Age at BMT	Female / 51 yrs	Male / 52 yrs	Male / 53 yrs
HLA mismatch	One DP	No	No
GVHD onset after BMT	Ten days	One month	Nine months
Hepatic disorders	One month after stopping IS	One month after stopping IS	Three months after stopping IS
	PT, 37 to 100%, AST 13 to 72 x, bilirubin 82 to 270 µmol/ L No hypergammaglobulinemia		

IS : Immunosuppression

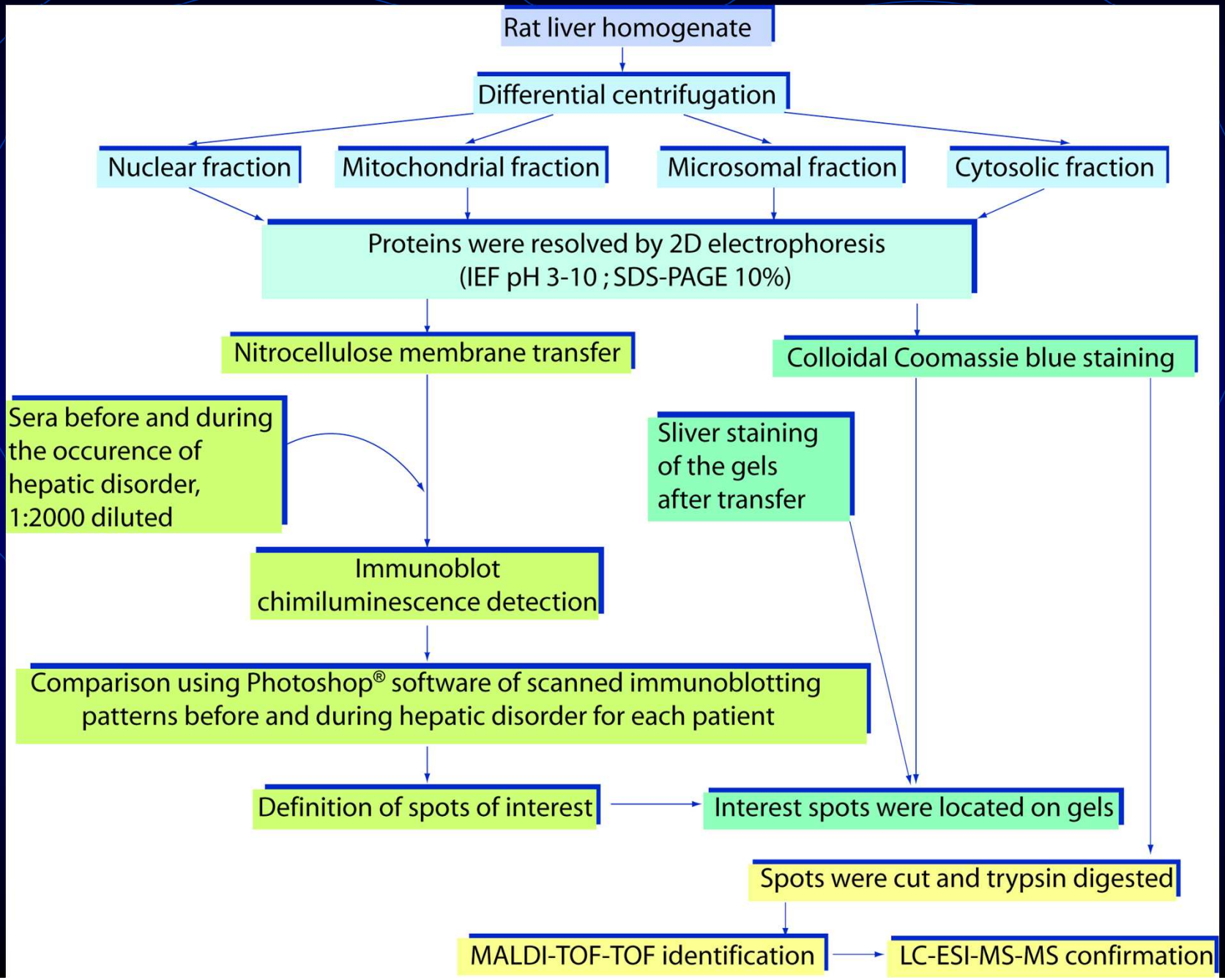
PATIENTS (2)

	Patient N°1	Patient N°2	Patient N°3
Viral serology	HCV positive before BMT, no HCV-RNA detection	HCV negative	HCV negative
	HAV, HBV negative. Herpes, CMV, EBV negative post BMT		
Antibody by indirect immunofluorescence (IIF)	Anti-nuclear negative before and at the onset of hepatitis	Anti-nuclear 1:80 at the occurrence of hepatic disorder	Anti-nuclear 1:640 at the occurrence of hepatic disorder
	Anti-smooth muscle, anti-LKM1, anti-mitochondria negative before and at the onset of hepatic disorder		
Histological features	Periportal and pericentrolobular necrosis + bridging necrosis in patients N°2 and N°3 . Abundance of lymphocytes and plasmocytes Fibrosis in patients N°2 and N°3		





METHODS

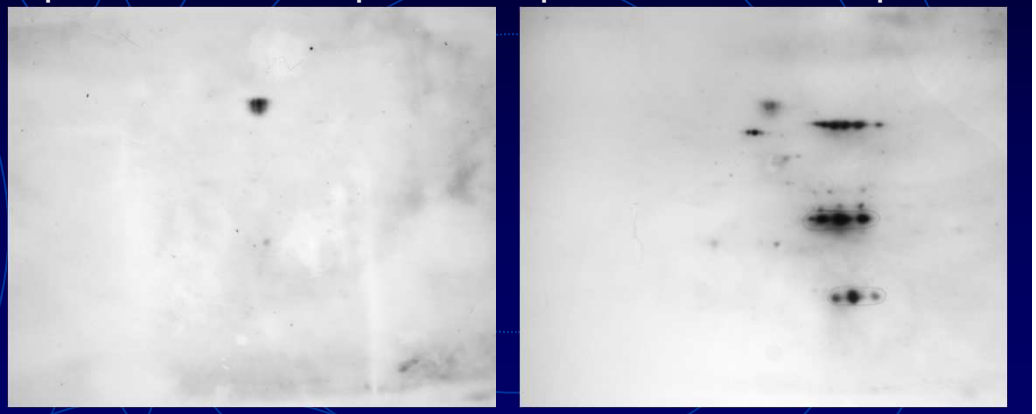

Rat liver homogenate
Differential centrifugation
Nuclear fraction
Mitochondrial fraction
Microsomal fraction
Cytosolic fraction
Proteins were resolved by 2D electrophoresis
(IEF pH 3-10 ; SDS-PAGE 10%)
Nitrocellulose membrane transfer
Colloidal Coomassie blue staining
Sera before and during the occurence of hepatic disorder, 1:2000 diluted
Sliver staining of the gels after transfer
Immunoblot chimiluminescence detection
Comparison using Photoshop® software of scanned immunoblotting patterns before and during hepatic disorder for each patient
Definition of spots of interest
Interest spots were located on gels
Spots were cut and trypsin digested
MALDI-TOF-TOF identification
LC-ESI-MS-MS confirmation

RESULTS (1)

✓ Spots more numerous and more intensely expressed than those stained by sera before the hepatic disorder

Patient N°1, cytosolic fraction

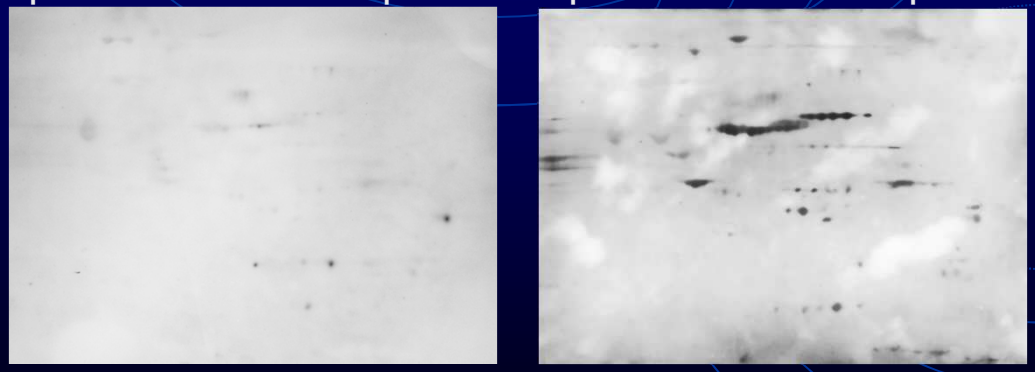
pI 3.0 pI 10.0 pI 3.0 pI 10.0



before *at the moment of hepatic disorder*

Patient N°2, microsomal fraction

pI 3.0 pI 10.0 pI 3.0 pI 10.0

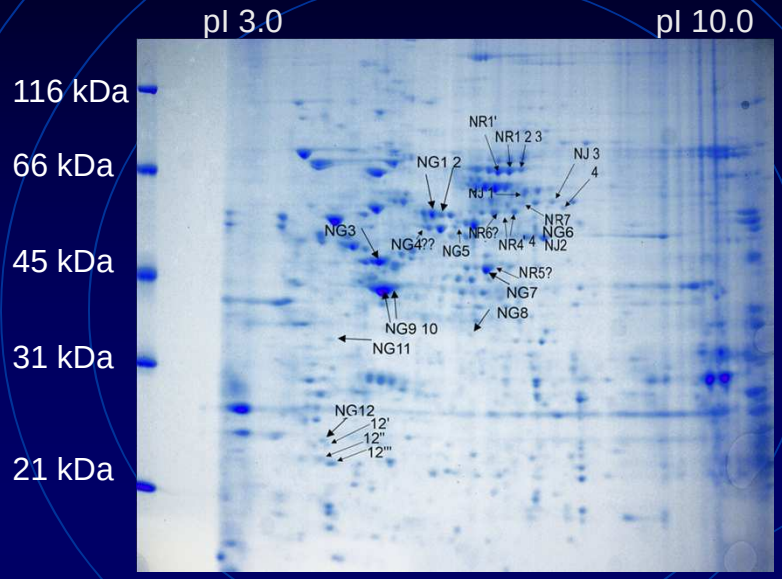


before *at the moment of hepatic disorder*

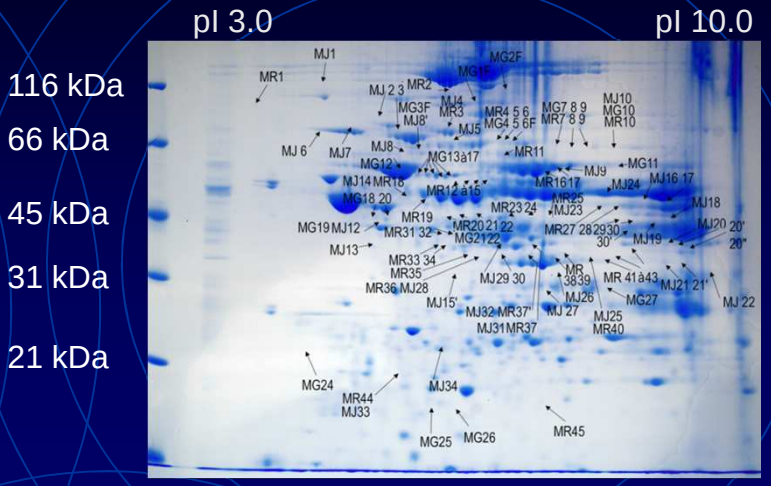
✓Great pattern heterogeneity depending on the patient

RESULTS (2)

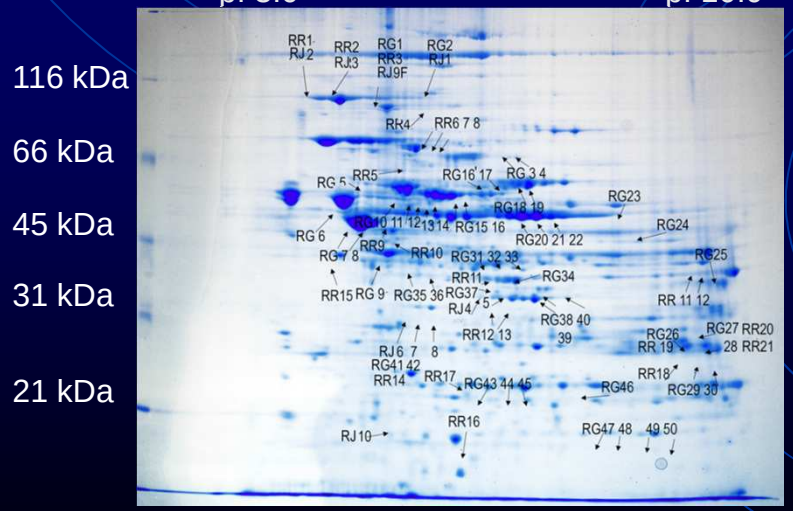
259 spots stained only by sera at the moment of the hepatitis



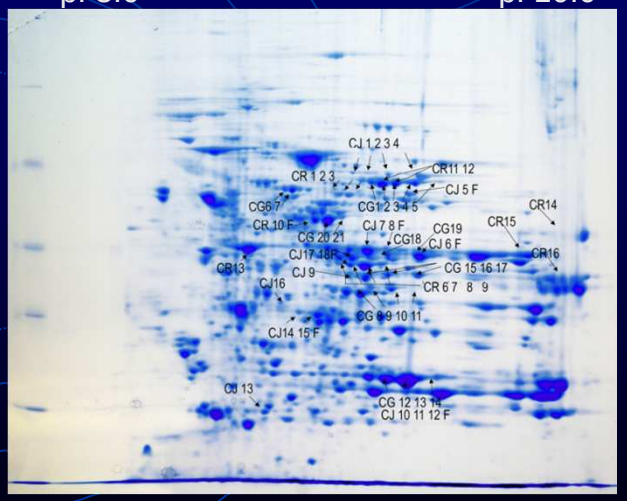
Nuclear fraction



Mitochondrial fraction



Microsomal fraction



Cytosolic fraction

RESULTS (3)

✓ 227 spots were actually identified

✓ Corresponding to 110 different proteins, some spots being isoforms of the same protein.

RESULTS (4)

13 COMMON PROTEINS STAINED BY THE 3 PATIENT SERA AT THE TIME OF HEPATIC DISEASE

- 60S acidic ribosomal protein P0
- Arginase 1
- Carboxylesterase 3
- Aldehyde dehydrogenase
- ATP synthase subunit alpha
- Catalase
- Dihydrolipoyllysine-residue acetyl transferase component of pyruvate dehydrogenase complex
- Hydroxy methyl glutaryl-CoA synthase
- Long-chain specific acyl-CoA dehydrogenase
- Medium-chain specific acyl-CoA dehydrogenase
- Very-long-chain specific acyl-CoA dehydrogenase
- Transitional endoplasmic reticulum ATPase (VCP)
- Ubiquinol cytochrome C reductase complex core protein 1

RESULTS (5)
Antigens in autoimmune diseases

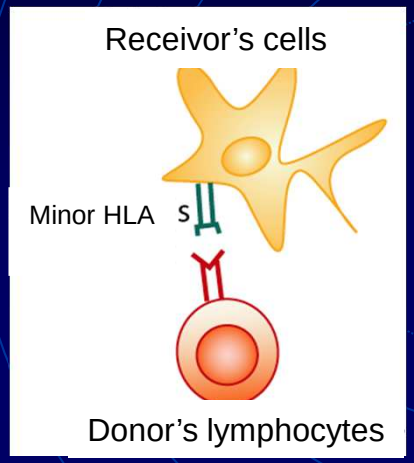
ANTIGEN	AUTOIMMUNE DISEASE
60S acidic ribosomal protein P0	Systemic lupus erythematosus
Arginase 1	Type 1 Autoimmune hepatitis
ATP synthase subunit alpha	Type 1 Autoimmune hepatitis
Catalase	Type 1 Autoimmune hepatitis
Dihydrolipoyllysine-residue acetyl transferase component of pyruvate dehydrogenase complex	Primary biliary cirrhosis
Transitional endoplasmic reticulum ATPase	Type 1 Autoimmune hepatitis

FROM GVHD TO AUTOIMMUNITY : Hypothesis

Acute GVHD

Tissue damage

Epiderm
Biliary duct
Gut epithelium



Alteration thymic epithelium
(without clinical translation)

Depletion of thymocytes
Destruction of dendritic cells
Destruction of thymic epithelial cells
Disappearance of Hassal bodies

Production of autoreactive T cells
Lack of LTreg specific of a self peptide

(Krenger W, 2008)

Cytokines, LPS, PAMPs

Stimulation innate immunity

?

Stimulation autoimmunity

(Lang K L, 2005)

Liberation of cryptic or modified Ags not recognized as self

Production of autoreactive T cells

(Teshima T, 2008)

CONCLUSION

❖ Description, similar to previous and rare reports, of a new type of *de novo* autoimmune hepatitis occurring after BMT.

❖ Autoantigens recognized by patient sera at the time of hepatic disorder are numerous.

❖ 13 proteins are common targets of antibodies present in all patient sera often described in hepatic diseases.

❖ Different from the ones found in *de novo* autoimmune hepatitis occurring after liver transplantation.

❖ The existence of a variant of GVHD can be put under discussion.

❖ This immune process leads to discuss mechanisms transforming an alloimmune reaction into an autoimmune response.

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