

PROTEÓMICA y SAF

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Acknowledgments



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PROTEOMIC

- **Protein separation methods (One-two-dimensional gel electrophoresis or mass spectroscopy)**
- **Detection methods using a non-specific staining**
- **Quantification by densitometry**
- **Validation of proteomic data by Western blot and quantitative real time RT-PCR**

OBJECTIVE

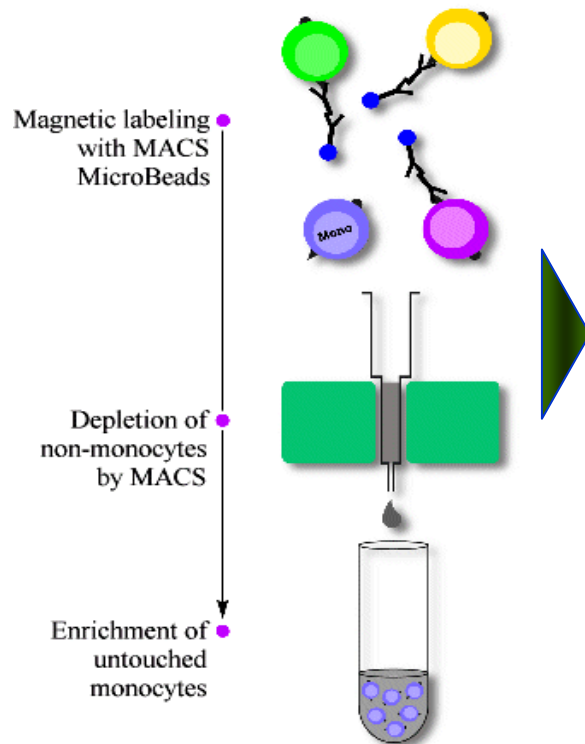
To analyze the expression of some proteins in monocytes of patients with APS which might be involved in the pathogenesis of thrombosis

PATIENTS CHARACTERISTICS

Variable	APS T (+) (n=32)	APS T (-) (n=19)	T aPL (-) (n=20)	Healthy (n=15)
F/M	23/9	19/0	12/8	9/6
Age	39±12	34±9	48±11	30±7
Venous T	13(41%)	0	10(50%)	0
Arterial T	20(63%)	0	10(50%)	0
Obstetric	3(9%)	19(100%)	0	0
aCL IgG	25(77%)	8(40%)	0	0
aCL IgM	17(54%)	11(55%)	0	0
LA	23(73%)	13(67%)	0	0

METHODS

Monocytes purification



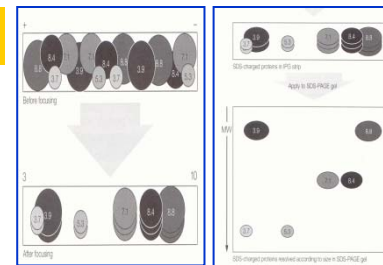
1

Cells lysates



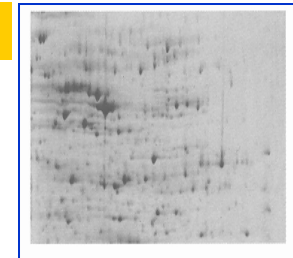
2

IEF- 2D electroforesis



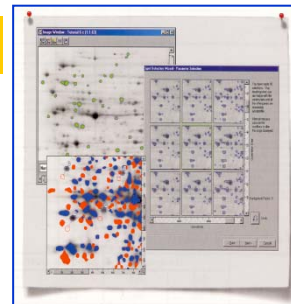
3

Staining: Silver MS/ compatible



4

Densit. Analysis PD-Quest 7.1.1



5

MALDI-TOF



6

Proteins analysis



7

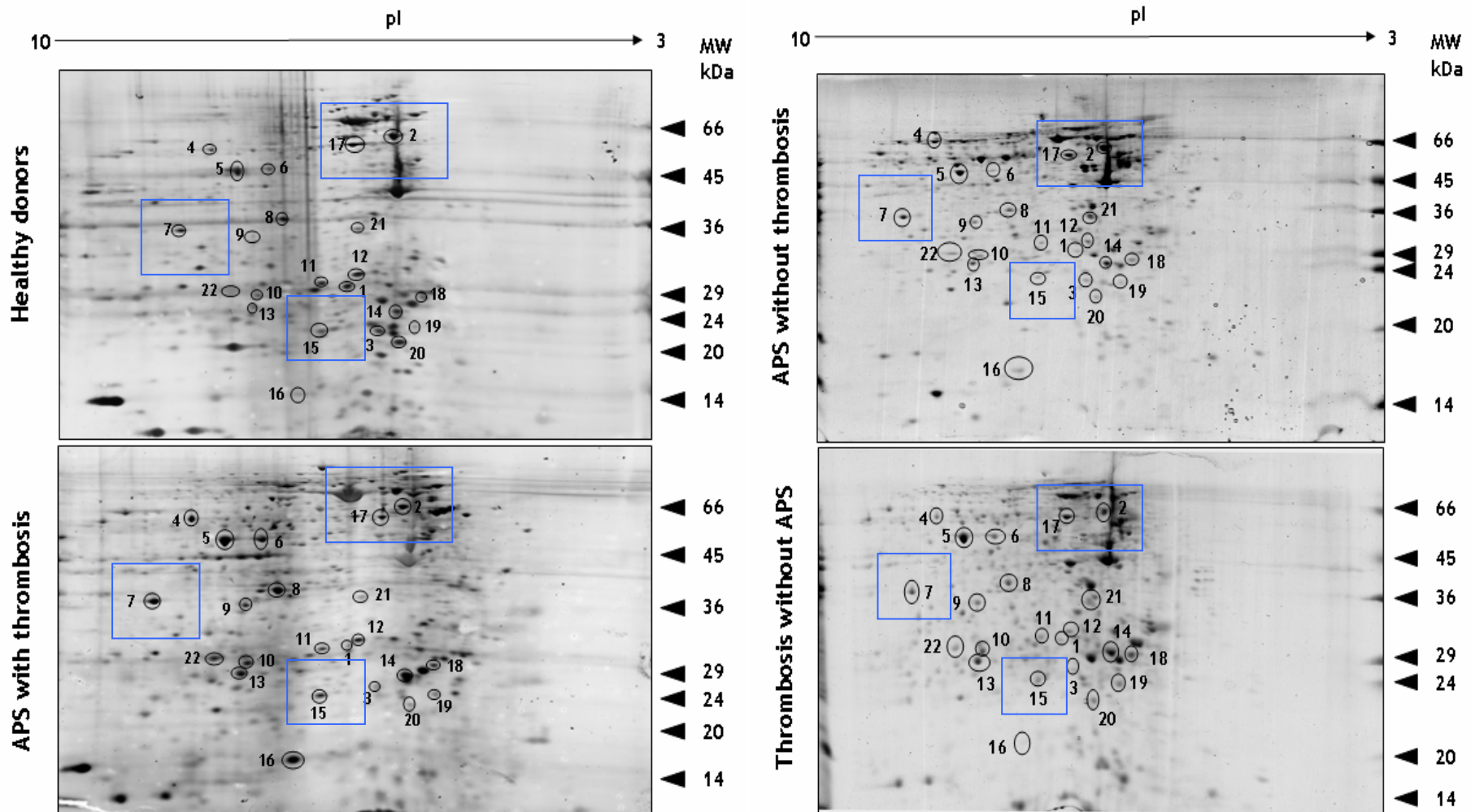
Confirmatory Analysis of relevant proteins : RT-PCR and Western blot

Protein Expression by Groups

Protein	APS T(+)	APS T(-)	T aPL(-)	CV
Anexin I	180±37	97±20	113±5	22%
Anexin II	221±52	105±30	97±11	18%
Rho A	378±48	127±15	123±29	20%
Ubiquitin like nedd8	887±65	101±18	98±19	15%
PDI	23±6	89±3	93±10	12%
HSP 60KD	52±8	89±10	92±16	14%

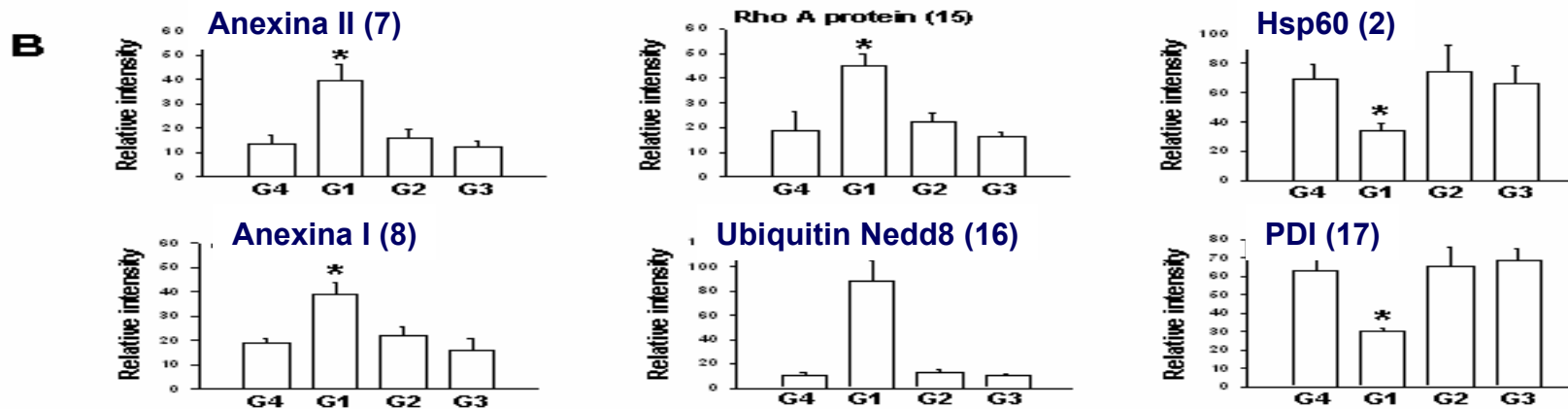
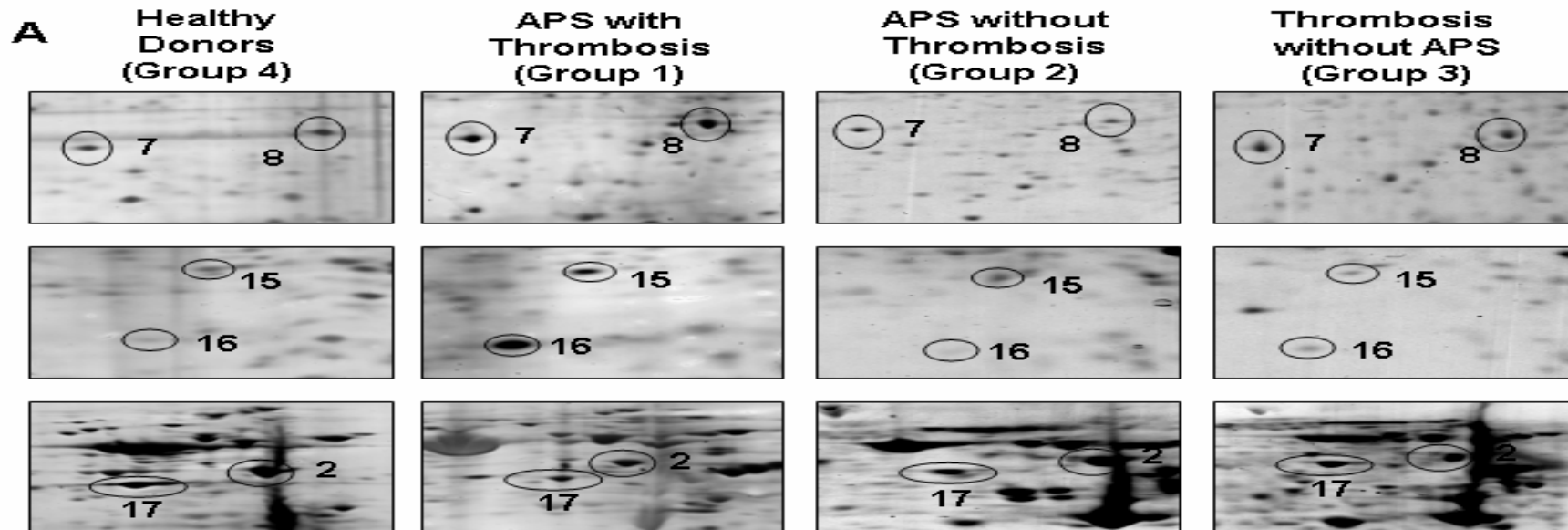
RESULTS

In vivo studies



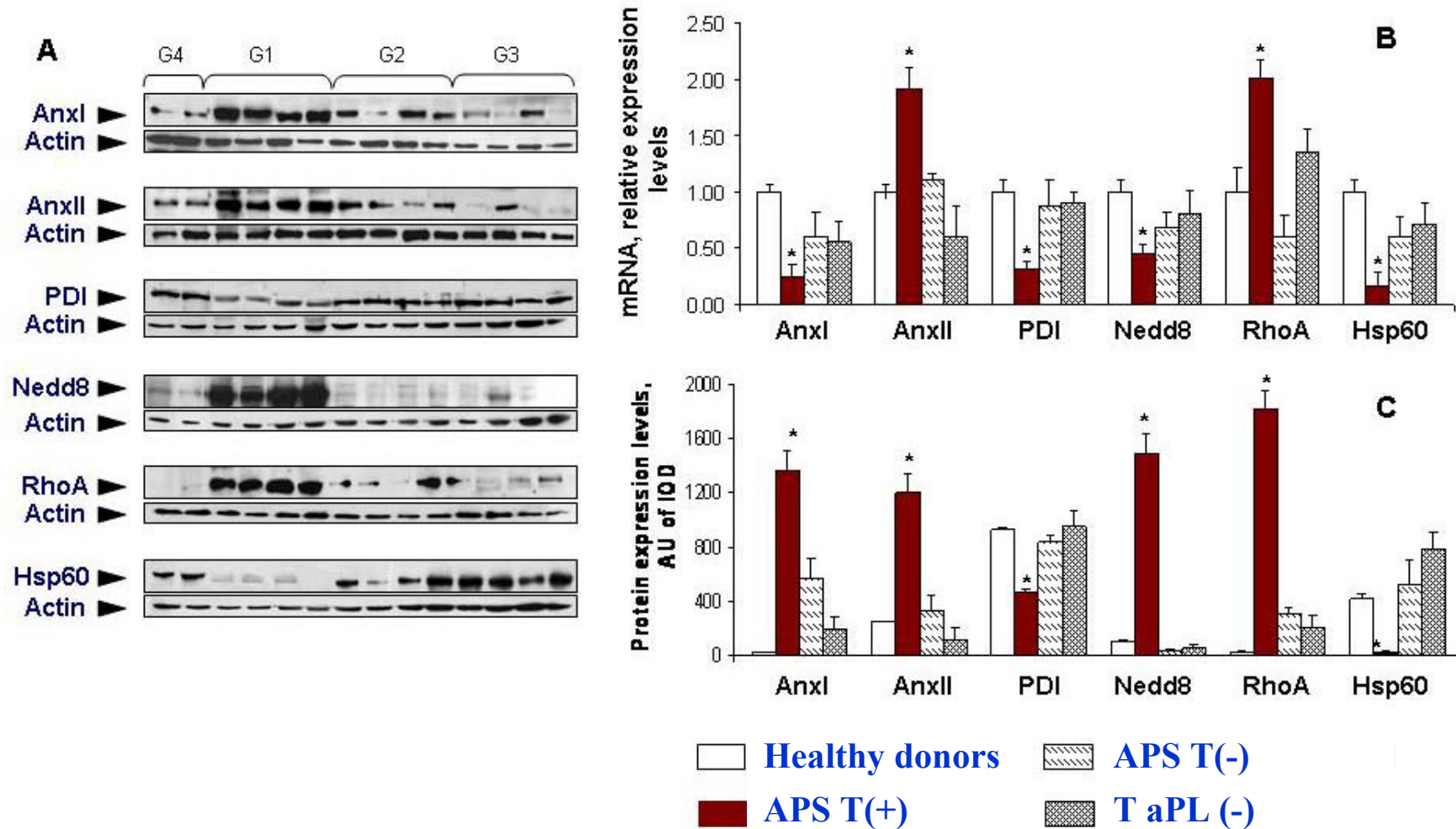
RESULTS

In vivo studies



RESULTS

Confirmatory analysis by Western blot and RT-PCR



Results: *In vitro* studies

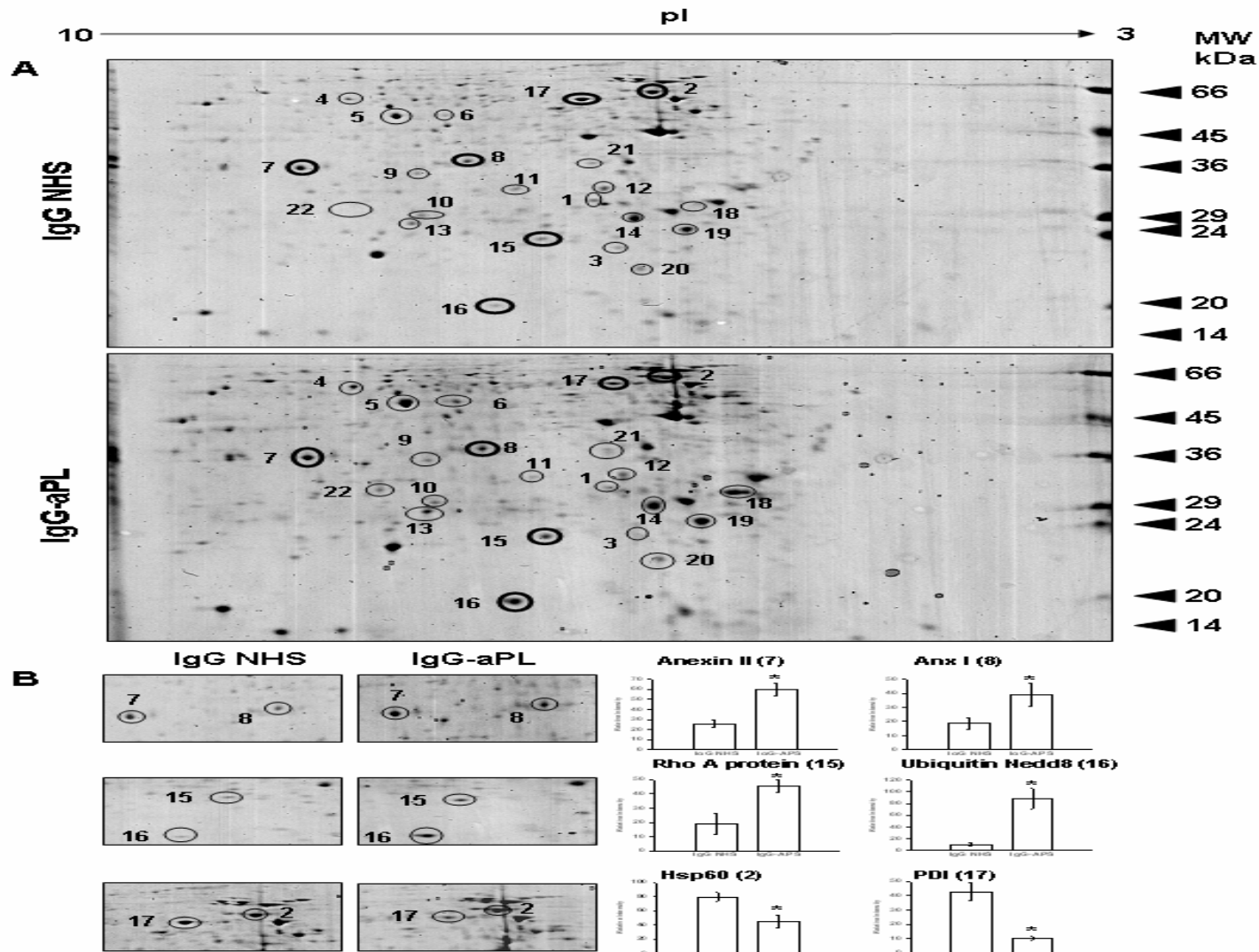


Figure 4

Summary

Upregulation

- **Anexin I**
- **Annexin II**
- **Ubiquitin nedd8**
- **Rho A**

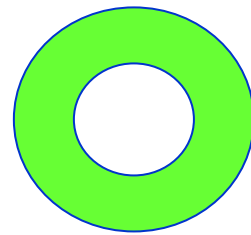
Downregulation

- **PDI**
- **HSP 60Kd**

Contribution to hypercoagulability

Tissue Factor

Activated
Monocyte



TF-VIIa $\xrightarrow{\text{IX} \rightarrow \text{IXa}}$ IXa VIIa

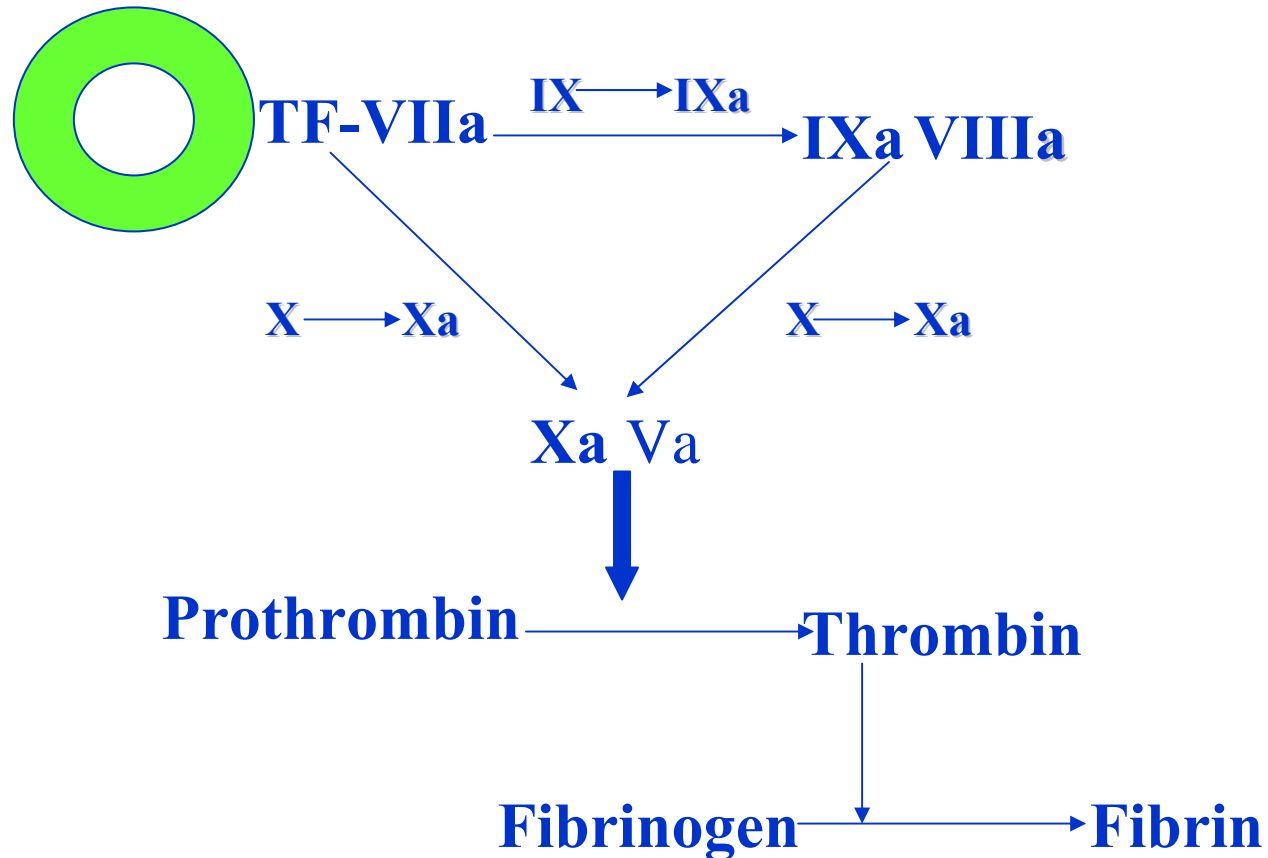
X \rightarrow Xa

X \rightarrow Xa

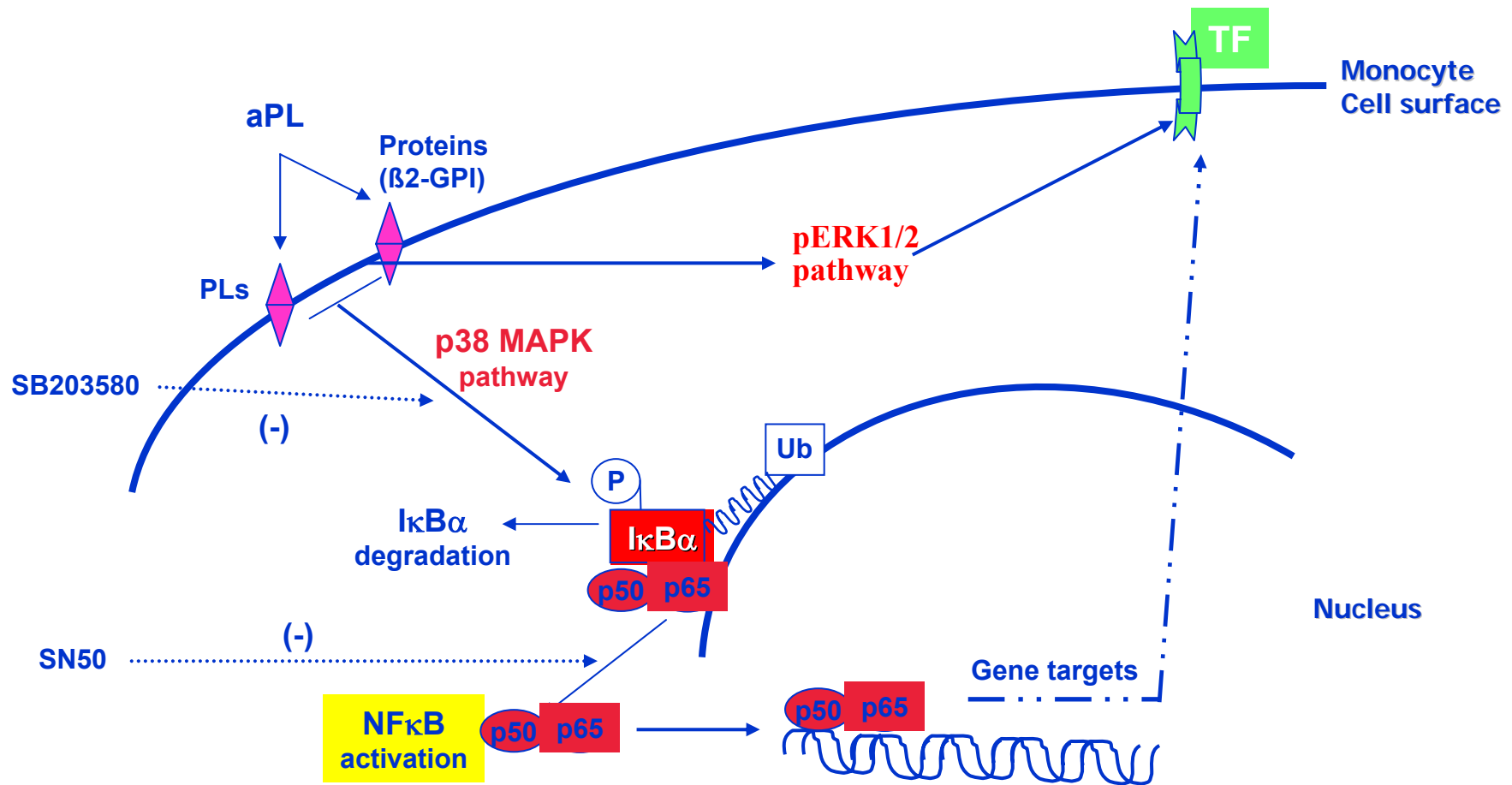
Xa Va

Prothrombin \rightarrow Thrombin

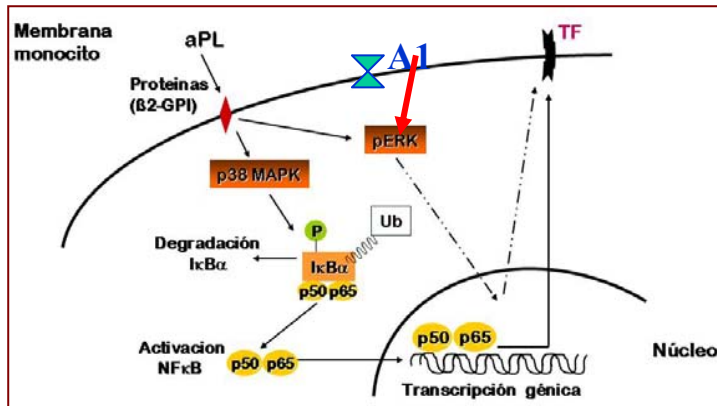
Fibrinogen \rightarrow Fibrin



Tissue Factor overexpression

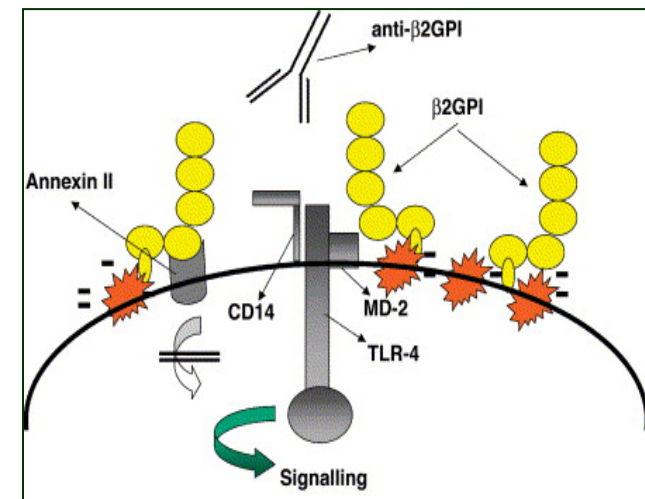


Contribution to hypercoagulability

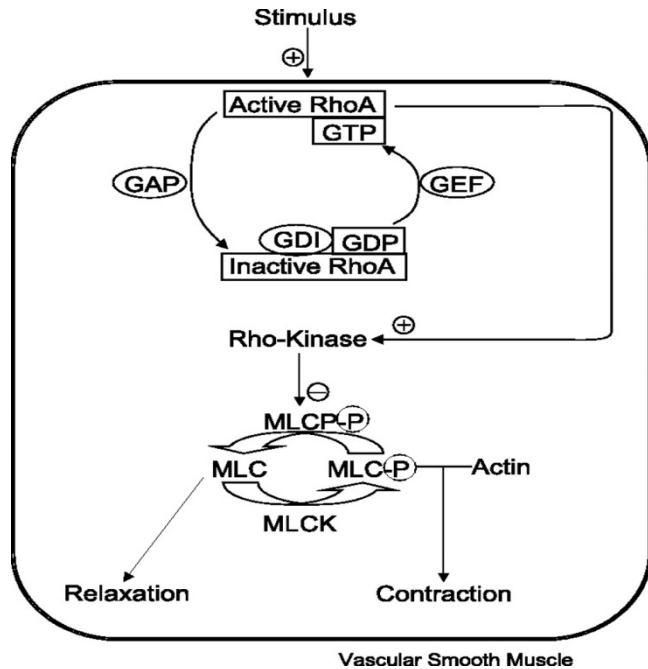


Increased expression of A1 leads to constitutive activation of ERK1/2 in RAW macrophages. We have found that the upregulation of A1 in APS monocytic cells was accompanied by constitutive activation of the MEK/ERK pathway. Involved in TF overexpression

A2 is a receptor for fibrinolytic activation. It has been demonstrated that binding of β_2 GPI to human umbilical vein endothelial cells is mediated by A2. It is a target for anti- β_2 GPI antibodies. Involved in TF overexpression

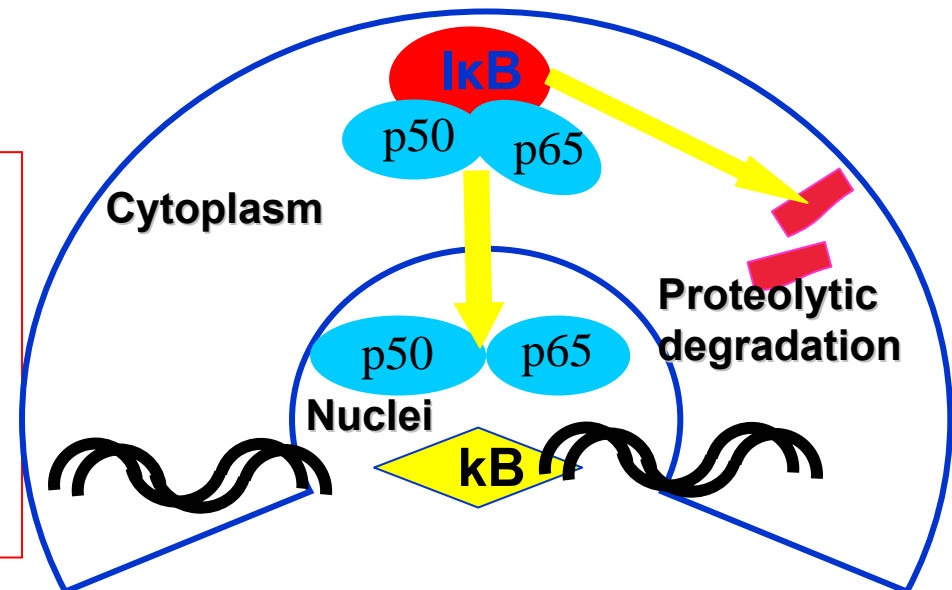


Contribution to hypercoagulability

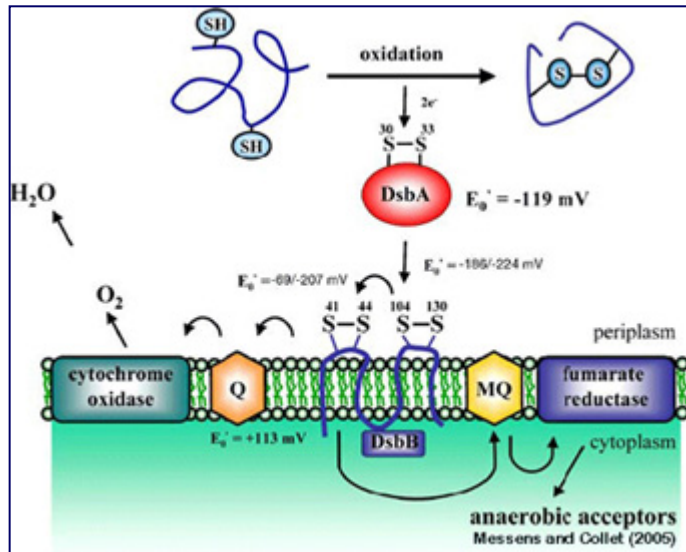


Rho A plays critical roles in inflammatory signal transduction cascades, such as those required for the activity of NFκB. In addition it has been demonstrated that inhibition of Rho/Rho kinase proteins downregulates the synthesis of TF.

Ubiquitin Nedd8 is involved in the proteolytic destruction of IκB (inhibitor of NFκB), which allows nuclear translocation of free NFκB, thus leading to activation of a multitude of target genes.

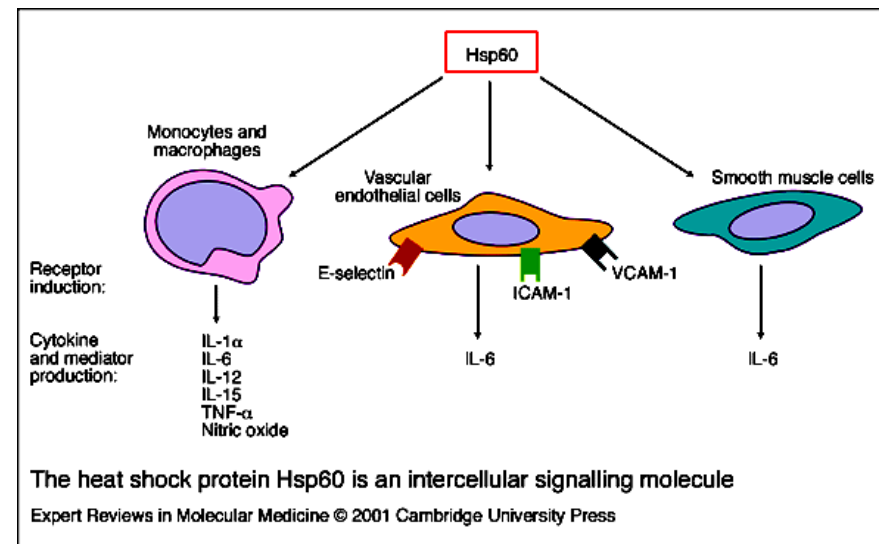


Contribution to hypercoagulability



PDI is associated with TF when coagulant activity is low and TF-VIIa signaling is enabled. Decreased PDI expression is associated with an increase in TF procoagulant activity

Hsp60 is present in the blood during inflammation, and has been found to be a target of autoantibodies and autoimmune T cells in healthy individuals, as well as those suffering from autoimmune diseases



Conclusions

- **This study has identified altered expression of proteins that might be directly related to thrombotic events in APS.**
- **It has also showed that all proteins necessary for monocyte-induced procoagulant activity are specifically altered in their expression in response to aPL.**
- **The different protein-expression patterns identified in patients with and without thrombosis might define different subgroups of APS patients.**

Next Step

- **Study differences in protein expression in subgroup of APS patients (arterial vs venous vs obstetric APS)**
- **Prospective study to investigate if protein pattern is predictive of phenotype/risk of events in aPL positive patients**