Repurposing Drugs for Neuroimmune Disease

March 13, 2014

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www.marconsultinginc.com

Here we are not afraid to follow the truth wherever it may lead,
Nor tolerate error so long as freedom is left to combat it
Thomas Jefferson

Why Study Drug Repurposing ?

➢ Take advantage of Knowledge gained from decades of drug development

- hematological malignancies
- HIV/AIDs
- HTLV associated inflammatory
- New “omic” technologies

➢ 3/11/14 FDA released draft guidance outlines the following key issues in drug development in CFS/ME:

• The case definitions or criteria for CFS/ME used to define a patient population in the context of drug development (??)

• Recommendations for establishing efficacy in CFS/ME based on patient-reported symptoms & measurements of exercise capacity!

A new drug discovered today would take 15-20 years to reach the patients

Much of current drug repurposing focuses on genotypic differences, an Equal focus should be on phenotypic screening and clinical observations

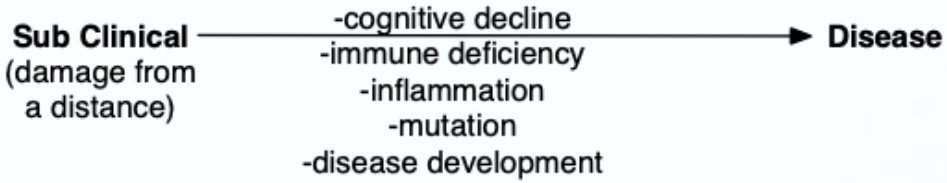
Phenotypic observations

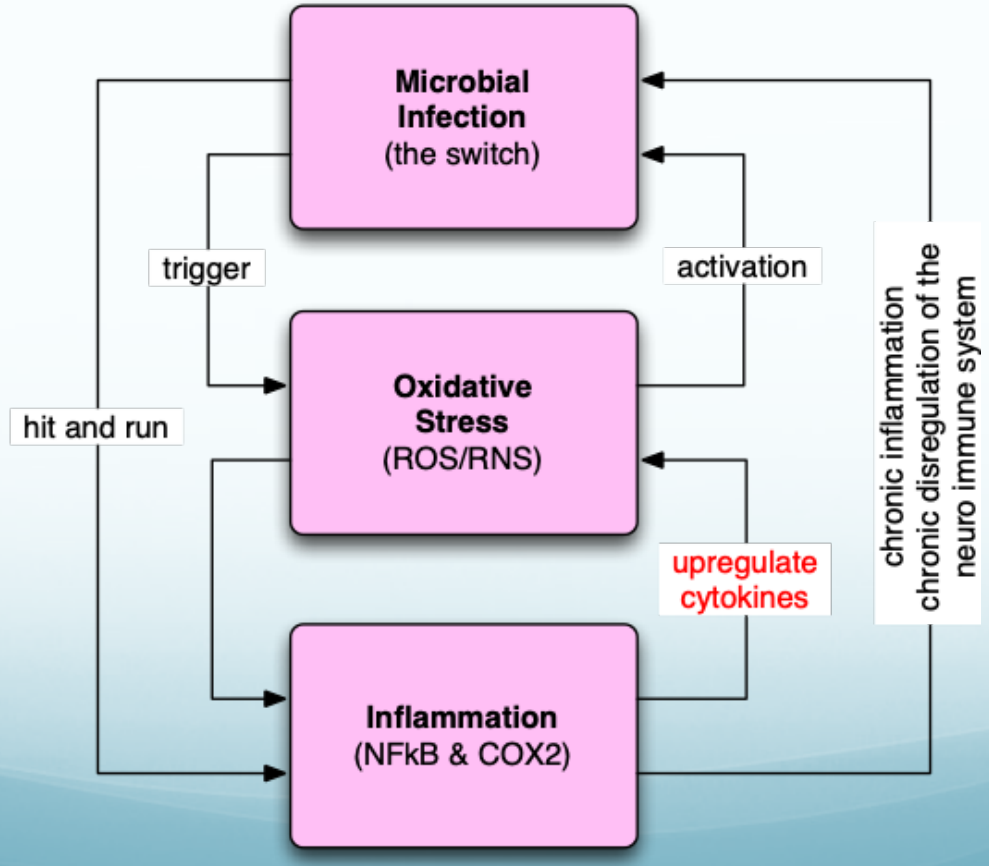
- 30% of 400 compounds profiled show new beneficial biology
- Up to 90% of new indications are driven by “on-target” activities
- Biology is complex and there is a tremendous amount that is not understood
- Phenotypic screening provides an opportunity to identify new clinically relevant uses of existing molecules driven by action on known molecular targets

Outline of Presentation

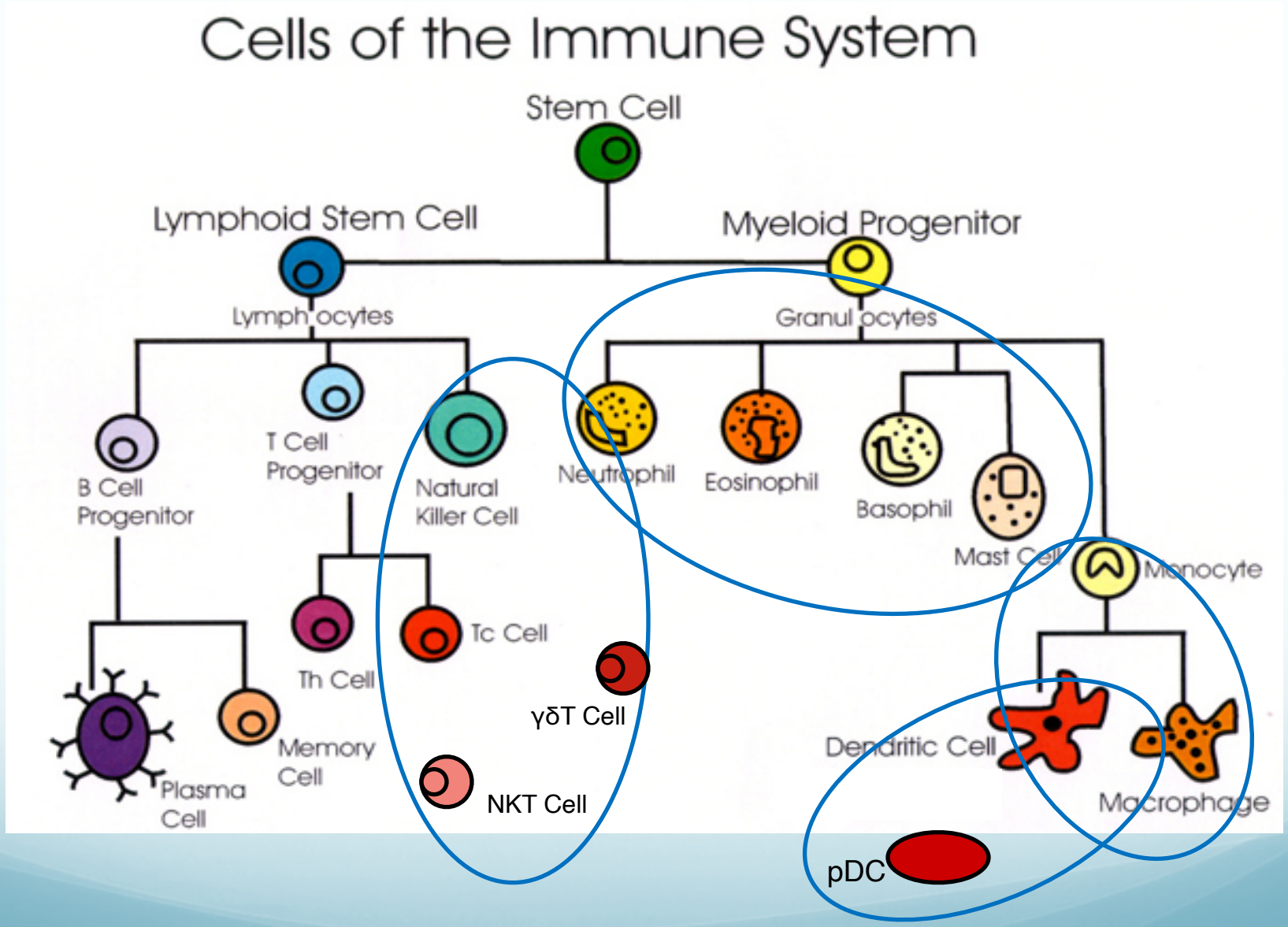
- **Abnormal Cellular Immunity in the Peripheral Blood of Patients With Neuroimmune Disease and Cancer (2013PRT)**
- **Review published and unpublished data of the immune abnormalities in neuroimmune disease, which suggest rationale for use of existing therapies**
- **Use the vast drug libraries, therapies knowledge from HIV-1 & HTLV-1 associated disease as well as hematological disease**

Key Contributors to Neuroimmune Disease Development





Innate Immune System –



Summary of Phenotypic Results

	ME/CFS, CLD vs. healthy
Total Cellularity	No Difference
CD45+ Leukocytes	No Difference
CD45+ Lymphocytes	Reduced in ME/CFS
CD45+ CD3- Lymphocytes	Reduced in ME/CFS
CD3+ CD56+ NKT cells	Increased in ME/CFS, CLD
CD3- CD56+ NK cells	Reduced in ME/CFS, CLD
$CD56^{DIM}$ NK subpopulation	Reduced in ME/CFS
$CD56^{DIM}$ CD16+ NK subpopulation	Reduced in ME/CFS
CD19+ B cells	Reduced in ME/CFS
CD19+ CD20+ CD23+ B subpopulation	Increased in ME/CFS
CD33+ CD14+ CD123- myeloid Population	Reduced in ME/CFS
Activated APCs	Increased in ME/CFS, CLD

Chronic innate immune activation leads to chronic immunosuppression

- Presence of CD20+ CD23+ B cells, not normally seen in healthy subjects, and activated APCs in some ME/CFS, CLD patients are similar to the myeloid and B cell defects described in HIV.
- The significant changes in the myeloid compartment including phenotypes are suggestive of activation of Antigen Presenting Cells (APCs, dendritic cells, monocyte/ macrophaes, microglia)
- Defective pDC function in ME/CFS
- Increased , γδT Cells clonality in ME/CFS, CLD, CLL, MCL
- Increased NKT compartment together with increased NK to NKT ratio.

Conclusion

Results Suggests a similar Disease cycle of chronic innate immune activation leading to an immune dysregulation and chronic immunosuppression and may guide future research towards the development of biomarkers and treatment targets

3 B-Cell Lines Derived Directly From CFS Patients' PBMCs

- CFS patient PBMCs were cultured; 3 samples developed into immortalized cell lines
- All three showed high CD20+ expression and two showed high CD23+ expression.
- All three showed strong similarity to B cells seen in patients.

Marker	MCL	WPI 1125	WPI 1186	WPI 1143
CD5	+	+	+	+
CD23	-	-	+	+
CD19	+	+	+	+
CD20	+	+	+	+
FMC7	+	+	-	-
CD3	-	-	-	-
CD4	-	-	-	-
CD7	+	-	-	-
CD8	-	-	-	-
CD10	-	-	-	-
CD38	+	+	+	+
CD45	+	+	+	+
CD56	-	-	-	-
CD122	-	-	-	-
HLA-DR	+	+	+	+
Lambda	+	+	-	-
Kappa	+	+	+	+

These Cell lines were developed from CFS patients. One, (1125) developed MCL; one (1186) was developed from a bone marrow biopsy, 3rd a CLL

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October 2011

PLoS one

Benefit from B-Lymphocyte Depletion Using the Anti-CD20 Antibody Rituximab in Chronic Fatigue Syndrome. A Double-Blind and Placebo-Controlled Study

Øystein Fluge[1]*, Ove Bruland[1,2], Kristin Risa[1], Anette Storstein[3], Einar K. Kristoffersen[4], Dipak Sapkota[1], Halvor Næss[3], Olav Dahl[1,5], Harald Nyland[3], Olav Mella[1,5]

1 Department of Oncology and Medical Physics, Haukeland University Hospital, Bergen, Norway, **2** Department of Medical Genetics and Molecular Medicine, Haukeland University Hospital, Bergen, Norway, **3** Department of Neurology, Haukeland University Hospital, Bergen, Norway, **4** Department of Immunology and Transfusion Medicine, Haukeland University Hospital, and The Gade Institute, University of Bergen, Bergen, Norway, **5** Institute of Internal Medicine, Section of Oncology, University of Bergen, Bergen, Norway

Abstract

Background: Chronic fatigue syndrome (CFS) is a disease of unknown aetiology. Major CFS symptom relief during cancer chemotherapy in a patient with synchronous CFS and lymphoma spurred a pilot study of B-lymphocyte depletion using the

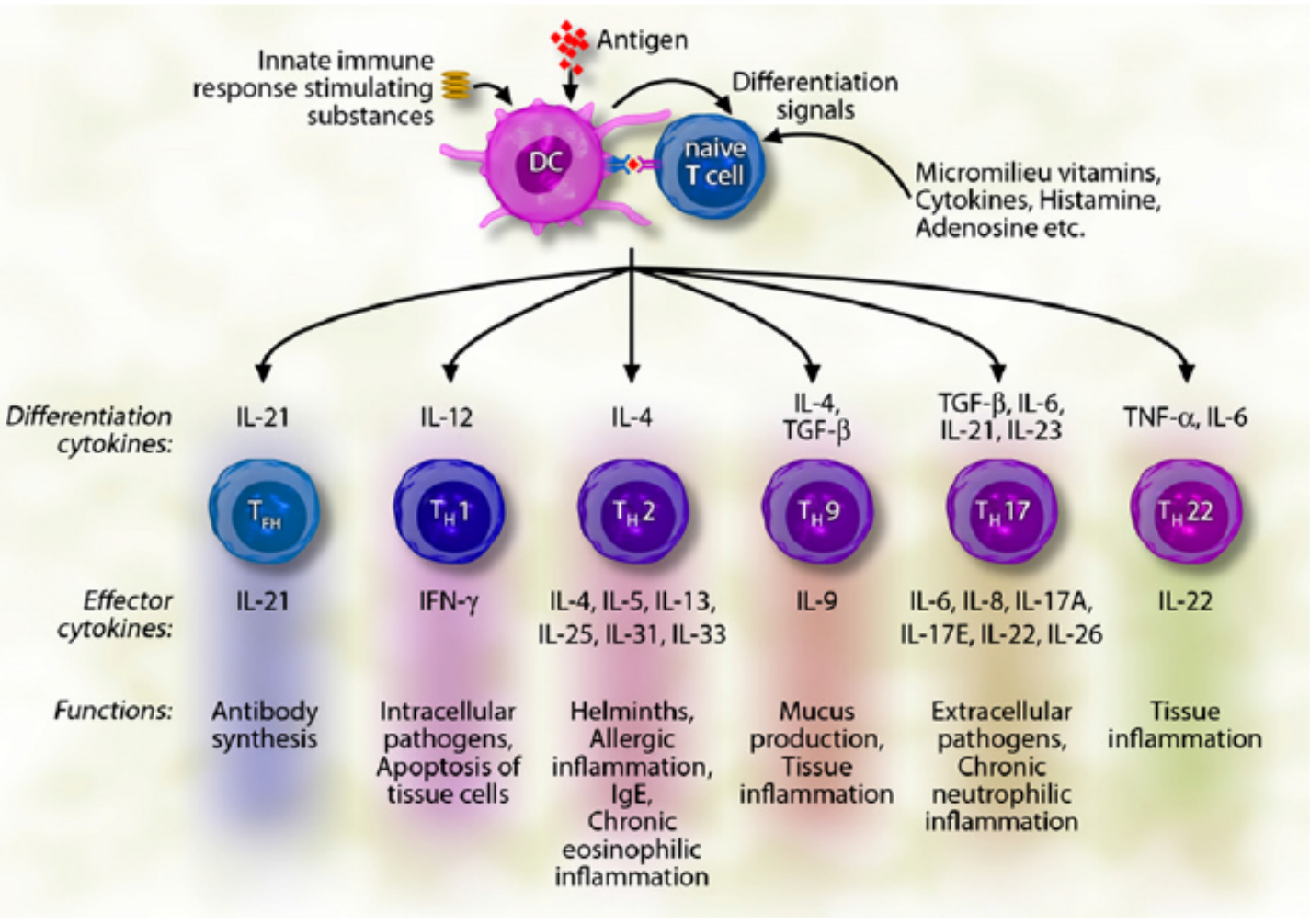
Original Article

Cancer, Dec 1 2012

Chronic Fatigue Syndrome and Subsequent Risk of Cancer Among Elderly US Adults

Cindy M. Chang, PhD, MPH[1]; Joan L. Warren, PhD[2]; and Eric A. Engels, MD, MPH[1]

Cytokine signatures can serve as a diagnostic fingerprint of pathogens and Biomarkers for therapy



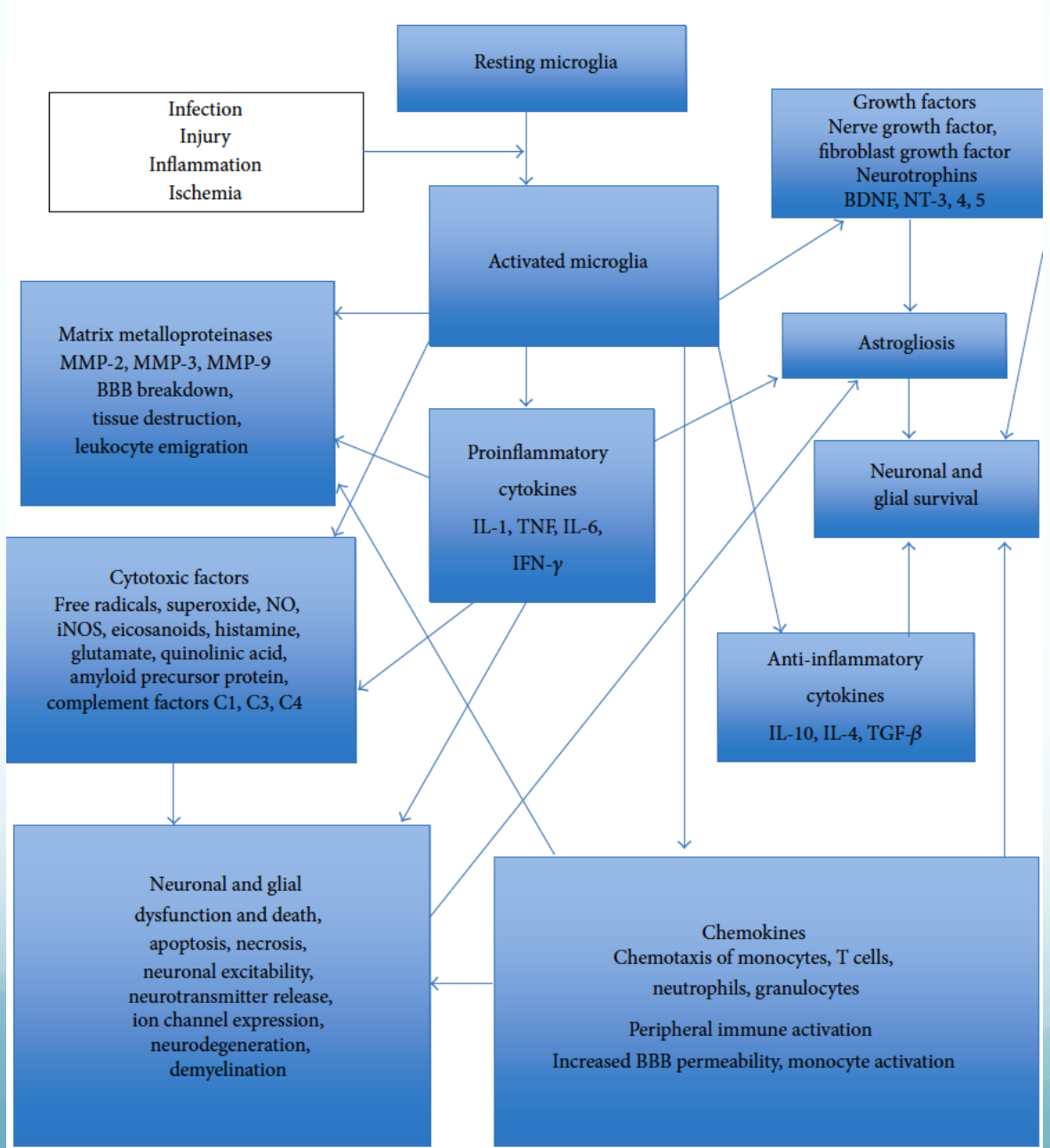
A Cytokine signature in a subgroup of CFS with active virus infections and B/T cell clonal expansions

CYTOKINES/ CHEMOKINES	Patient N = 118	Control N=138	P value	FUNCTION IN INFLAMMATION
IL-8	1045	13	<0.0001	RNase L and CMV activated
MIP-1α	763	91	0.0062	Elevated in Neurodegenerative disease
MIP-1β	1985	164	<0.0001	Elevated in Neurodegenerative disease
IL-6	336	29	<0.0001	Stimulates chronic inflammation
TNF-α	148	13	<0.0001	Stimulates chronic inflammation
IL1β	500	56	<0.0001	Stimulates chronic inflammation
IP-10	98	32	<0.0001	Interferon response protein
IFN-α	35	60	<0.0001	Stimulates macrophages and NK cells to elicit an anti-viral response
IL-13	28	86	<0.0001	Inhibits inflammatory cytokine production
IL-7	160	60	<0.0001	Stimulates proliferation of B and T lymphocytes and NK cells

Mean values in pg/ml: Red denotes up regulation, Blue denotes down regulation

in vivo 25: 307-314 (2011)

Central role of micgroglia in Neuroinflammation



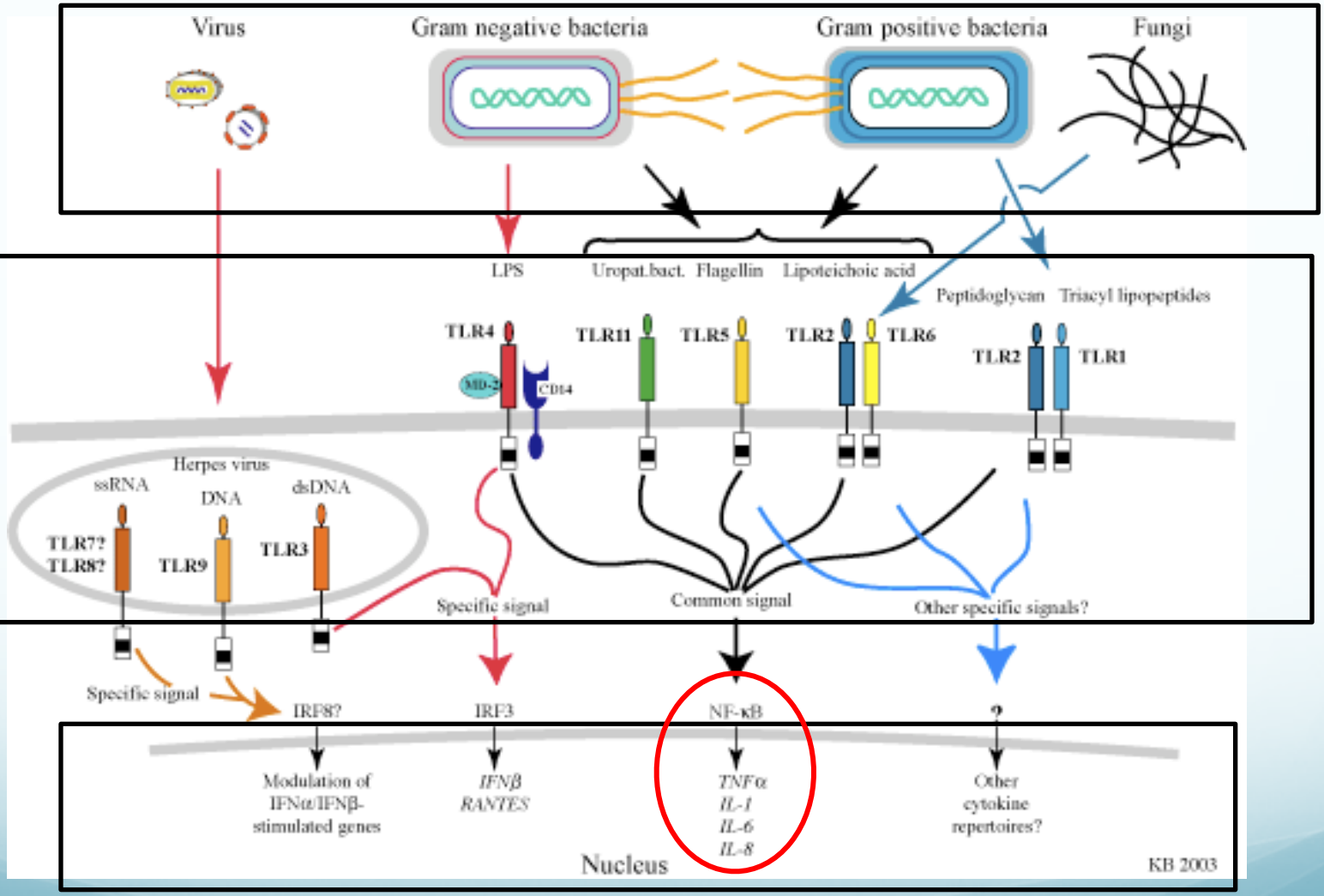
Rameshe Et. Al. 2013
Mediators of inflammation

Additional Cytokine Signatures may distinguish CLD and ME/CFS

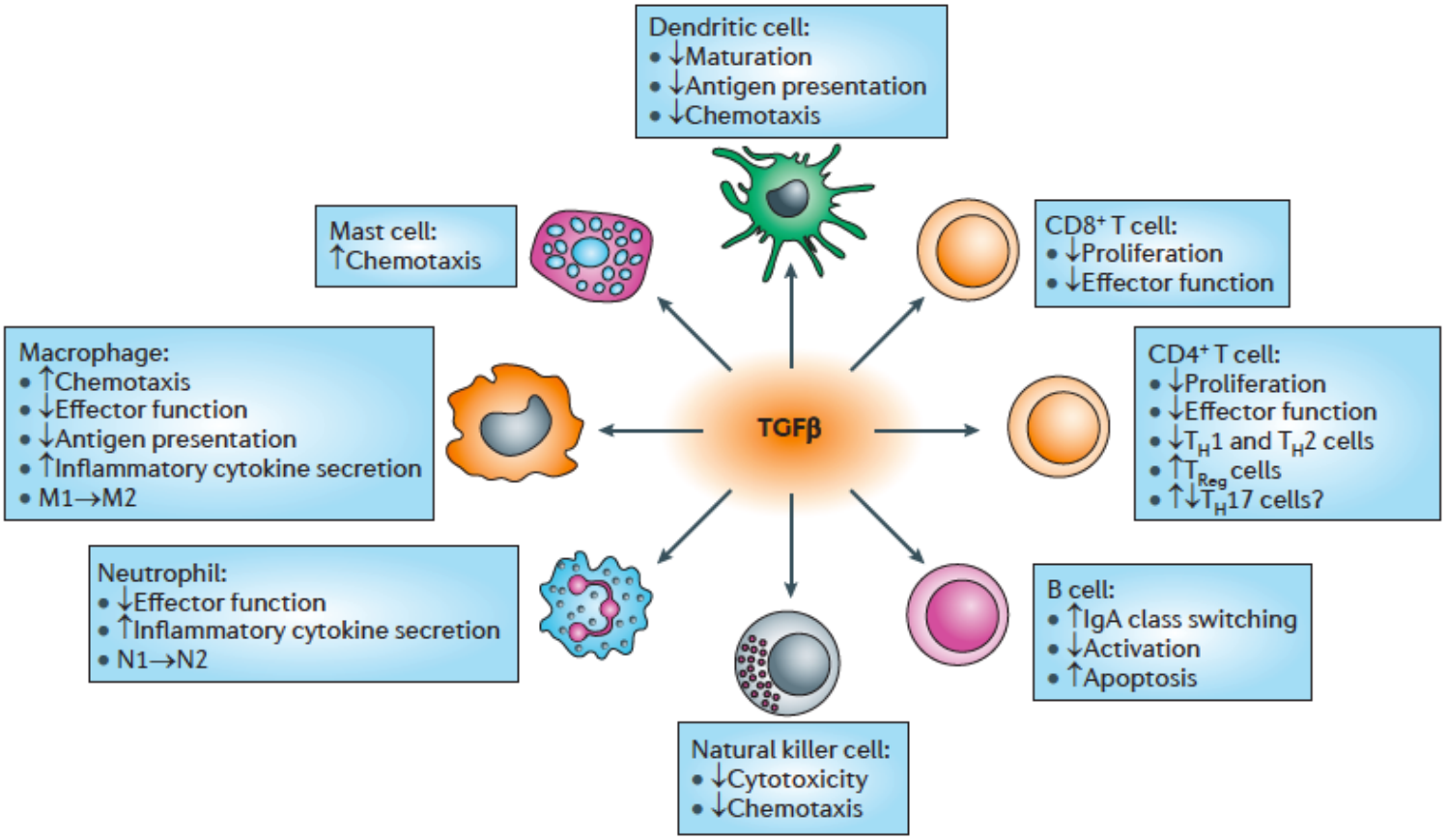
➢ Key cytokines in those signatures provide footprints for pathogen identity And immune dysregulation contributing to disease

- IL-9 and TH9 clones
- TGFβ

Inhibition of NFkB Prevents Cytokine Storm induced by certain Pathogens

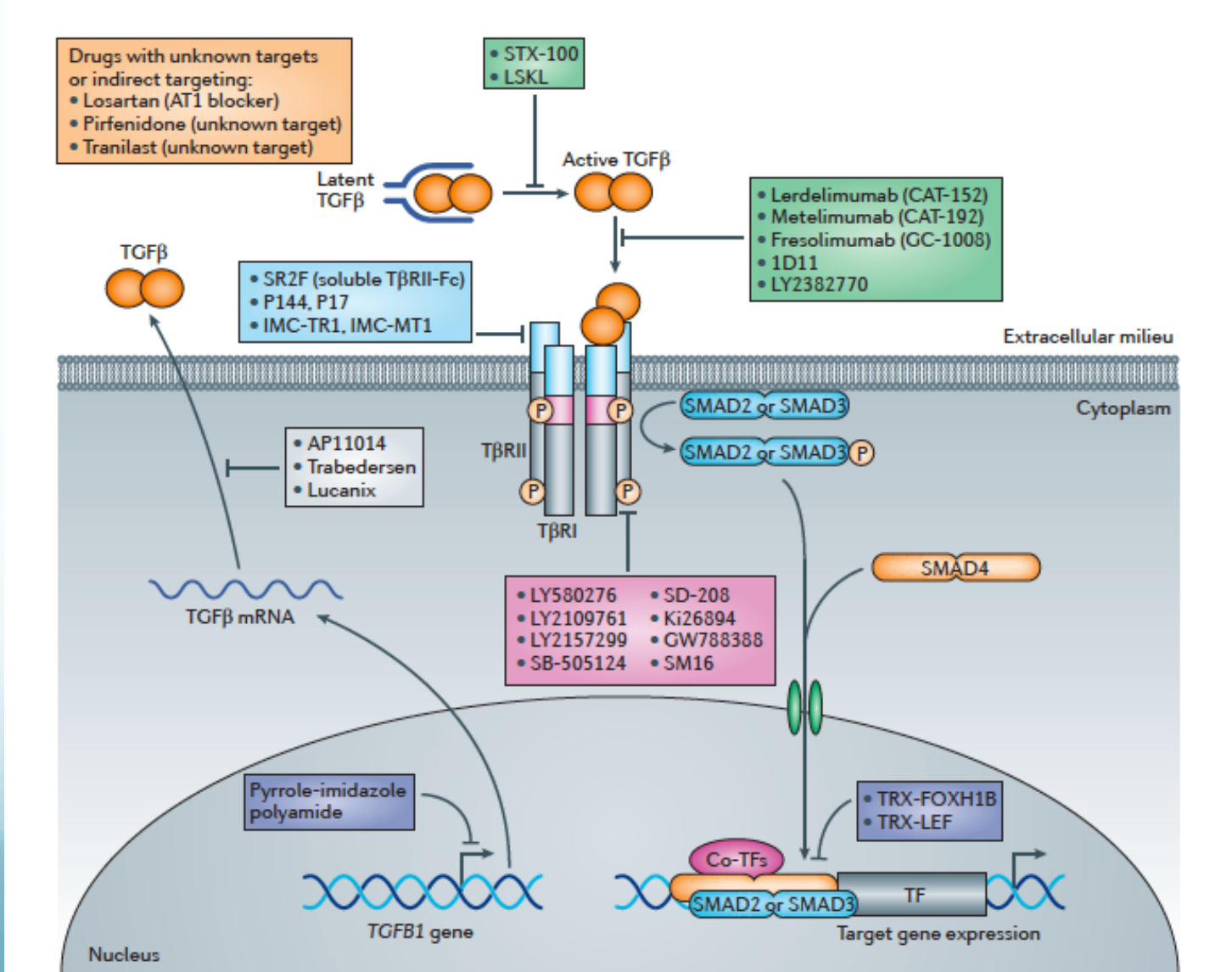


TGFβ effects on Immune Cells

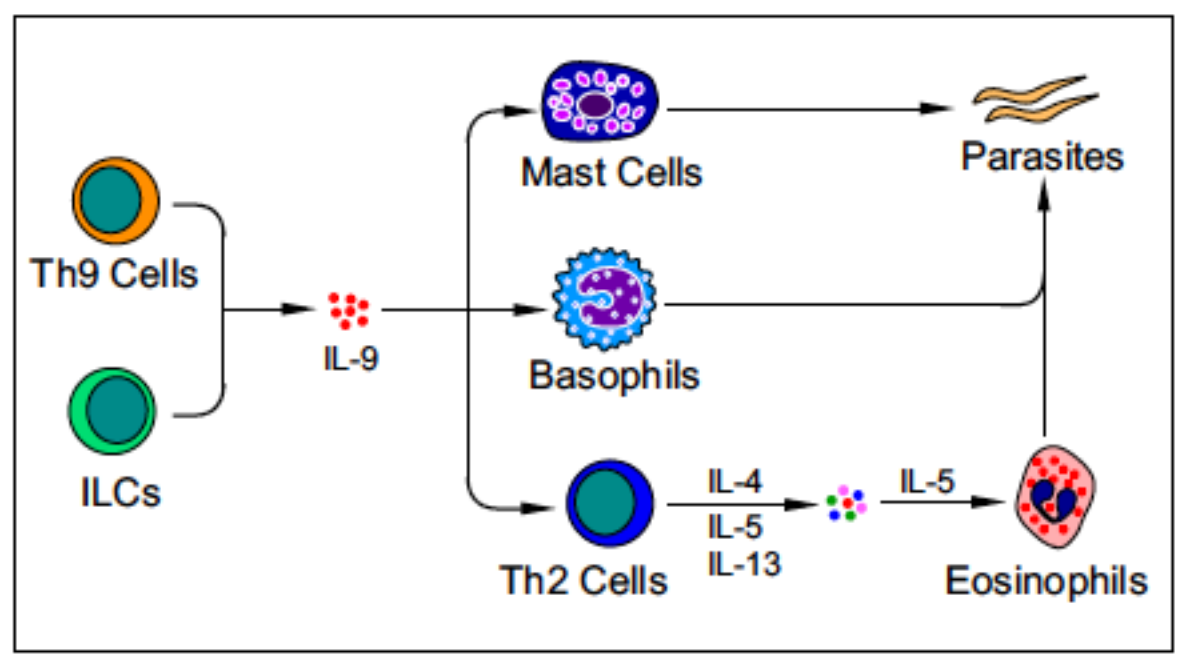


Nature Reviews Akhurst & Hata October 2012

Therapeutic approaches to blocking TGFβ



The Role of IL9 in T_H2 Immunity



nature medicine

Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis

Aurélien Trompette[1], Eva S Gollwitzer[1], Koshika Yadava[1], Anke K Sichelstiel[1], Norbert Sprenger[2], Catherine Ngom-Bru[2], Carine Blanchard[2], Tobias Junt[3], Laurent P Nicod[1], Nicola L Harris[4] & Benjamin J Marsland[1]

- Fatty acid produced from High fiber diet boost your immune system
- provides evidence supporting the use of butyrate as therapy for inflammatory bowel diseases like Crohn's disease
- molecular basis for the role of butyrate on the production of Treg Cells

Cell Host & Microbe
Short Article

Gut Dysbiosis Promotes M2 Macrophage Polarization and Allergic Airway Inflammation via Fungi-Induced PGE_2

Yun-Gi Kim,[1,2,5] Kankanam Gamage Sanath Udayanga,[1,2] Naoya Totsuka,[1,2] Jason B. Weinberg,[4] Gabriel Núñez,[5] and Akira Shibuya[1,2,3,*]
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[2]Japan Science and Technology Agency, Core Research for Evolutional Science and Technology (CREST)
[3]Life Science Center of Tsukuba Advanced Research Alliance (TARA)
University of Tsukuba, Tsukuba, Ibaraki 305-8575, Japan
[4]Department of Pediatrics and Communicable Diseases, Microbiology, and Immunology
[5]Pathology and Comprehensive Cancer Center
University of Michigan Medical School, Ann Arbor, MI 48109, USA
*Correspondence: ashibuya@md.tsukuba.ac.jp
http://dx.doi.org/10.1016/j.chom.2013.12.010

Celebrex-originally identified to block PGE2 induced inflammation

Only certain antibiotic promote fungal overgrowth in the gut, suggesting Specific commensal bacteria have the ability to prevent colonization of Candida

JLB

Review

CD57$^+$ T lymphocytes and functional immune deficiency

Daniele Focosi,[*,1] *Marco Bestagno,*[†] *Oscar Burrone,*[†] *and Mario Petrini**

*Division of Hematology, Azienda Ospedaliera Santa Chiara, University of Pisa, Pisa, Italy; and †Molecular Immunoogy Lab, International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy

RECEIVED AUGUST 23, 2009; REVISED SEPTEMBER 16, 2009; ACCEPTED SEPTEMBER 18, 2009. DOI: 10.1189/jlb.0809566

Utility of CD57 to measure functional immune deficiency in patients

- autoimmue disease
- Infectious disease
- cancers

OPEN ACCESS Freely available online

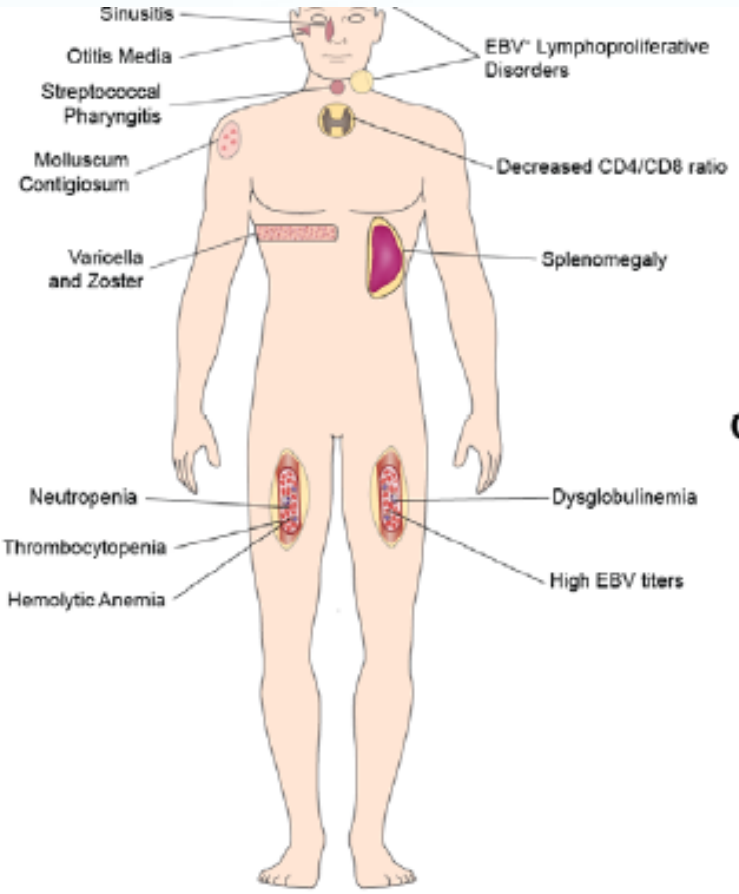
PLOS | ONE

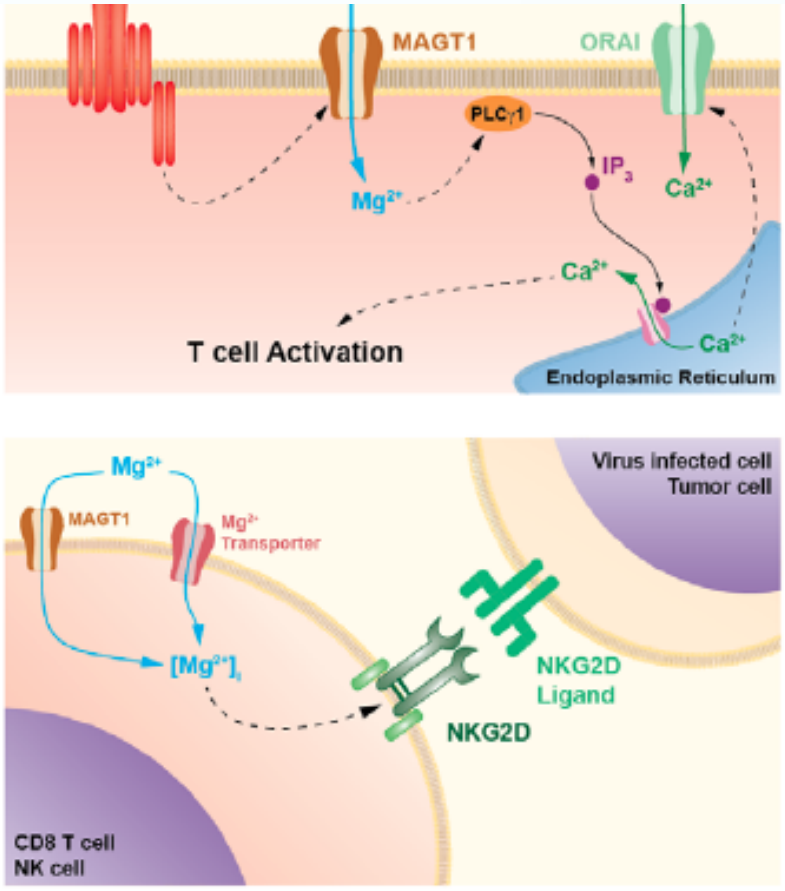
Deficient EBV-Specific B- and T-Cell Response in Patients with Chronic Fatigue Syndrome

Madlen Loebel[1]*, Kristin Strohschein[1,2], Carolin Giannini[1], Uwe Koelsch[3], Sandra Bauer[1], Cornelia Doebis[4], Sybill Thomas[1], Nadine Unterwalder[3], Volker von Baehr[4], Petra Reinke[5,6], Michael Knops[1], Leif G. Hanitsch[1], Christian Meisel[1,3], Hans-Dieter Volk[1,5], Carmen Scheibenbogen[1,5]

1 Institute for Medical Immunology, Charité University Medicine Berlin, Campus Virchow, Berlin, Germany, **2** Julius Wolff Institute, Charité University Medicine Berlin, Campus Virchow, Berlin, Germany, **3** Labor Berlin GmbH, Immunology Department, Charité University Medicine Berlin, Campus Virchow, Berlin, Germany, **4** Institute for Medical Diagnostics, Berlin, Germany, **5** Berlin-Brandenburg Center for Regenerative Therapies (BCRT), Charité University Medicine Berlin, Germany, **6** Department Nephrology, Charité University Medicine Berlin, Germany

XMEN- New Primary Immune Deficiency





First PID associated with specific loss of NKG2D expression
Rituximab
Magnesium threonate supplementation

APDS- a new PID

Science 342:866 (2013

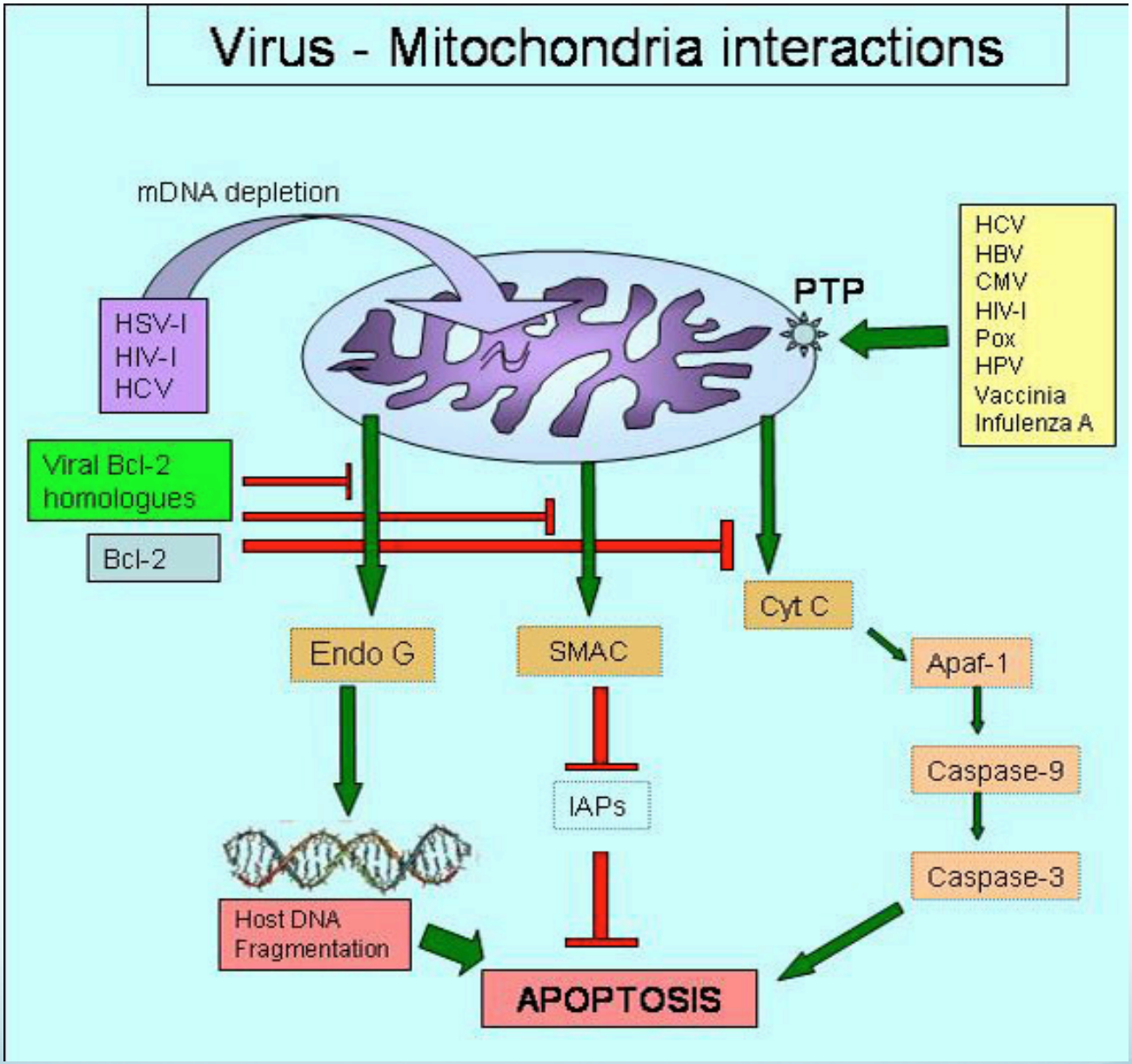
Phosphoinositide 3-Kinase δ Gene Mutation Predisposes to Respiratory Infection and Airway Damage

Ivan Angulo,[1]* Oscar Vadas,[2]*† Fabien Garçon,[3]* Edward Banham-Hall,[3]* Vincent Plagnol,[4] Timothy R. Leahy,[5,6] Helen Baxendale,[7] Tanya Coulter,[6,8] James Curtis,[1] Changxin Wu,[1] Katherine Blake-Palmer,[1] Olga Perisic,[2] Deborah Smyth,[9] Mailis Maes,[1] Christine Fiddler,[1] Jatinder Juss,[1] Deirdre Cilliers,[10] Gašper Markelj,[11] Anita Chandra,[7] George Farmer,[12] Anna Kielkowska,[13] Jonathan Clark,[13] Sven Kracker,[14,15] Marianne Debré,[16] Capucine Picard,[15,16,17] Isabelle Pellier,[18] Nada Jabado,[19] James A. Morris,[20] Gabriela Barcenas-Morales,[21] Alain Fischer,[14,15,16] Len Stephens,[3] Phillip Hawkins,[3] Jeffrey C. Barrett,[20] Mario Abinun,[5] Menna Clatworthy,[1] Anne Durandy,[14,15,16,17] Rainer Doffinger,[7] Edwin R. Chilvers,[1] Andrew J. Cant,[5] Dinakantha Kumararatne,[7] Klaus Okkenhaug,[3] Roger L. Williams,[2] Alison Condliffe,[1]‡ Sergey Nejentsev[1]‡§

Characterized by:

- recurrent respiratory infections
- increased circulating Transitional B Cells
- Increased IgM
- Decreased IgG
- Impaired responses to vaccines

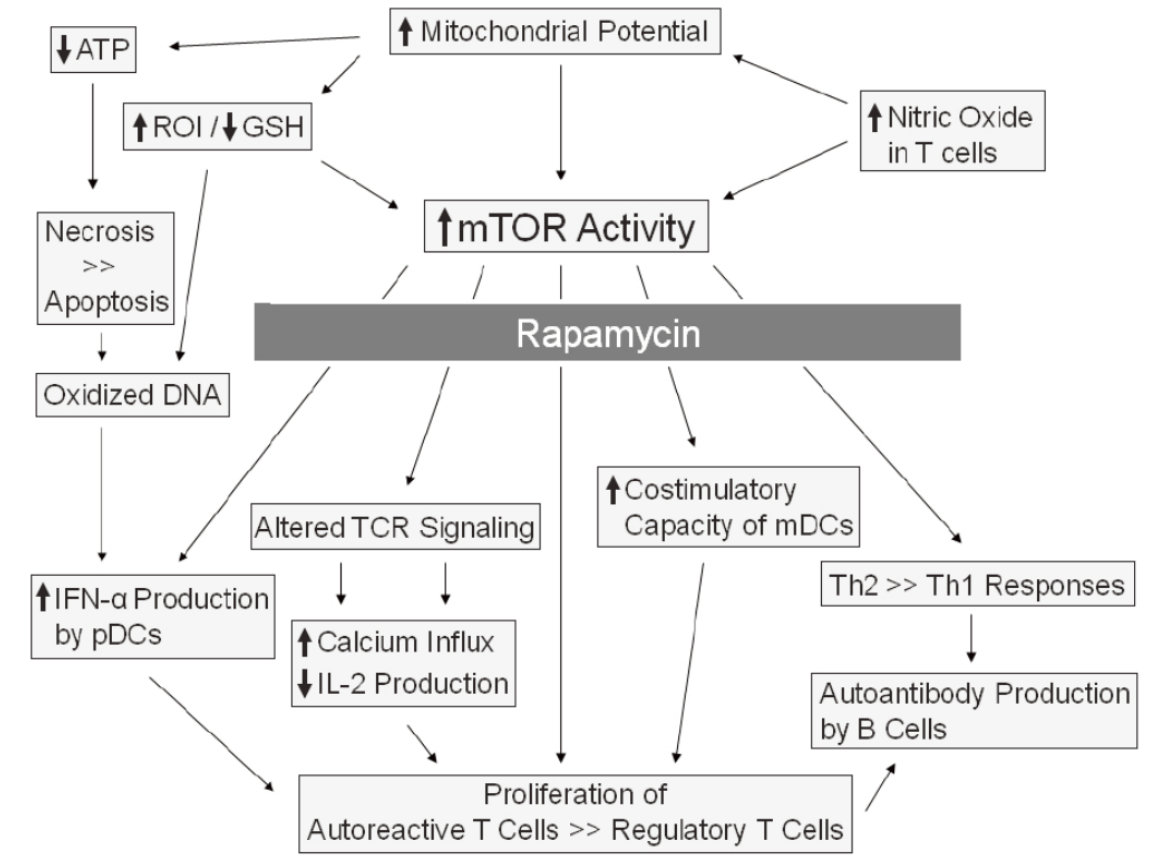
. Selective p110δ inhibitors IC87114 and GS-1101 reduced the activity of the mutant enzyme in vitro, which suggested a therapeutic approach for patients with APDS.



Recent advance in genomic technologies have identified ~1000 nuclear genes that regulate mitochondrial function ...

mTOR a master regulator of Neuroimmune Disease?

http://www.discoverymedicine.com/David-Fernandez/files/2010...



Ramesh et al

Yates *et al. Acta Neuropathologica Communications* 2013, **1**:3
http://www.actaneurocomms.org/1/1/3

ACTA NEUROPATHOLOGICA
COMMUNICATIONS

RESEARCH **Open Access**

Dysfunction of the mTOR pathway is a risk factor for Alzheimer's disease

Sharon C Yates[1], Amen Zafar[1], Paul Hubbard[1], Sheila Nagy[1], Sarah Durant[2], Roy Bicknell[2], Gordon Wilcock[3], Sharon Christie[3], Margaret M Esiri[4], A David Smith[5] and Zsuzsanna Nagy[1*]

New Technologies provide new opportunities for drug repurposing: Comprehensive Sequence Analysis of Nuclear mitochondrial genes

- NGS for variants in the nuclear mitochondrial exome that contribute to neurological disorders whose symptoms resemble mitochondrial disease.

Case Reports In CFS patients Results:

- Abnormal autosomal dominant Variant was found in SCN4A gene that is likely a pathological mutation
- Pathological mutations found in two other patients also with multiple functional conditions (ME/CFS)

- Drugs targeting channelopathies (Diamox)
- mitochondrial targets mTOR (Rapamycin)
- apoptosis

Germline RNASEL variants ME/CFS, CLD and Cancer

Incidental finding:

This patient has three variants in *RNASEL*. Mutations in this gene have been associated with predisposition to prostate cancer and this gene is a candidate for the hereditary prostate cancer 1 (HPC1) allele. One of these variants, p.E265*, has been reported in the literature in 4 brothers with prostate cancer.

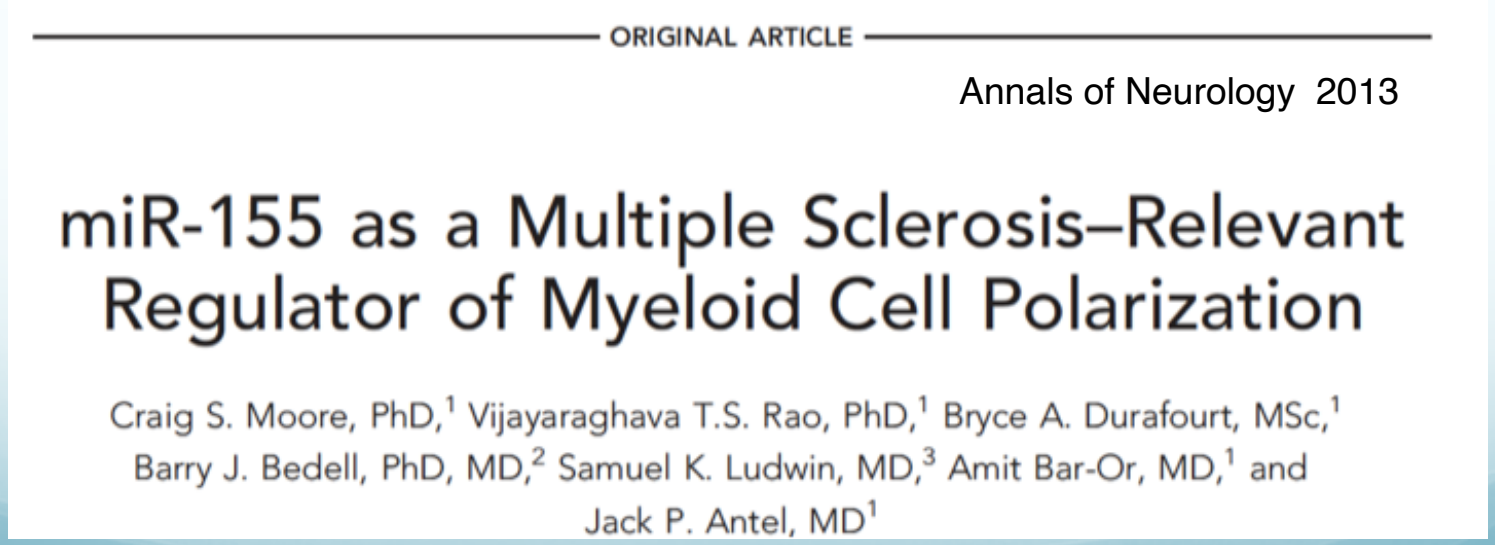
PLOS | ONE

Germline Mutation in RNASEL Predicts Increased Ris Neck, Uterine Cervix and Breast Cancer

Bo Eskerod Madsen, Eliana Marisa Ramos, Mathieu Boulard, Katarzyna Duda, Jens Overgaard, Marianne Nord
Lise Lotte Hansen

miR-155 a new target in ME/CFS?

- miR-155 has distinct expression profiles
- Plays crucial role in various physiological and pathological processes such as haematopoietic lineage differentiation, immunity, inflammation, cancer, and cardiovascular diseases miR-155 has been implicated in chronic DNA viral infections
- Been implicated in viral infections, particularly in those caused by DNA viruses.



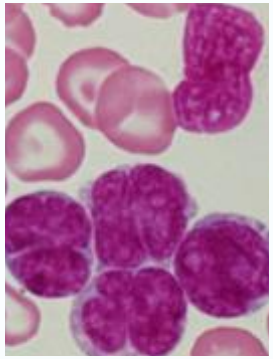
ORIGINAL ARTICLE

Annals of Neurology 2013

miR-155 as a Multiple Sclerosis–Relevant Regulator of Myeloid Cell Polarization

Craig S. Moore, PhD,[1] Vijayaraghava T.S. Rao, PhD,[1] Bryce A. Durafourt, MSc,[1] Barry J. Bedell, PhD, MD,[2] Samuel K. Ludwin, MD,[3] Amit Bar-Or, MD,[1] and Jack P. Antel, MD[1]

Pathogenesis of HTLV-I



- **Adult T cell leukemia**
  - Clonal malignancy of $CD4^+$ T cells.
  - Long latency; neonatal transmission
  - Immune deficiency

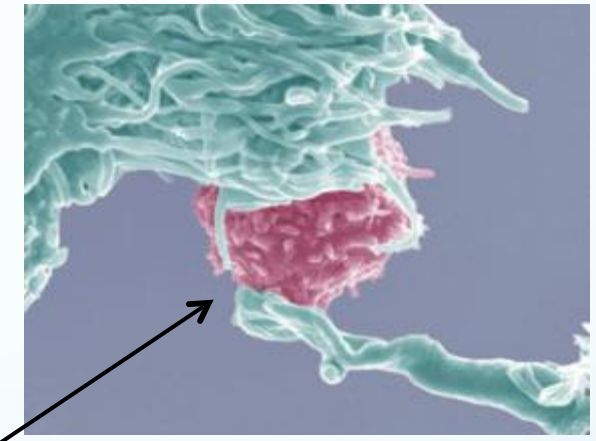
- **Prolonged survival/remission can be obtained by treatment with IFN-α and AZT**

- **Inflammatory syndromes:**
  - HTLV-I associated myelopathy/ Tropical spastic paraparesis
  - uveitis
  - arthropathy

- **Asymptomatic in majority of individuals:**
  - HTLV-I carriers: 5-8% lifetime risk of developing disease

Dendritic Cells vs. Viruses

- Dendritic cells- potent antigen-presenting cells:
  - Play a central in immune responses against viruses
  - Located at sites of viral entry
    - Mucosal membranes
    - Peripheral blood
- 2 types of DC in peripheral blood:
  - Myeloid dendritic cells (conventional DC)
    - classical APCs
    - initiate the activation of T cells
  - Plasmacytoid dendritic cells
    - innate immune response (IFN-α)
    - Link innate and adaptive immunity

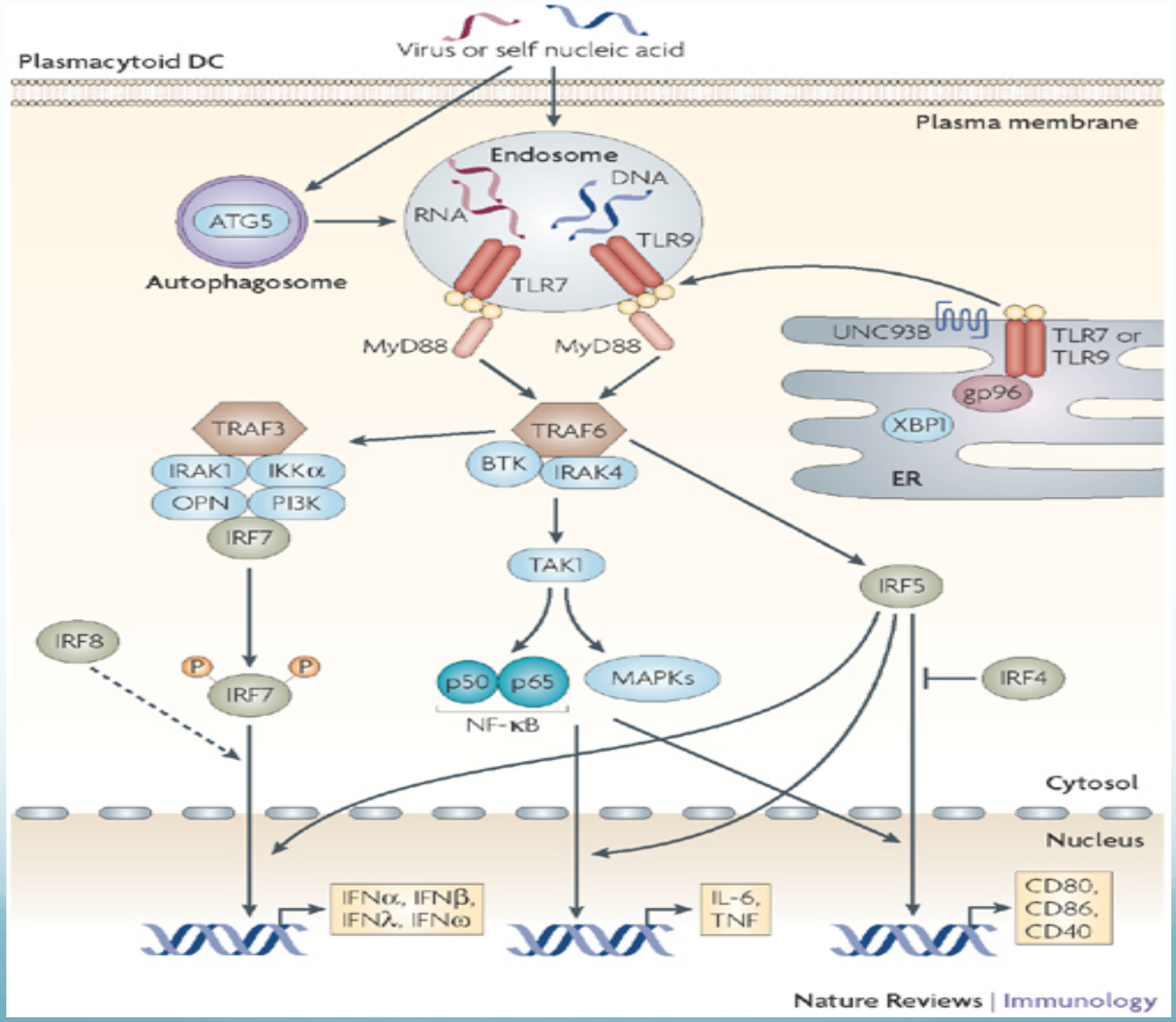


O. Schwartz;
Nat Cell Bio

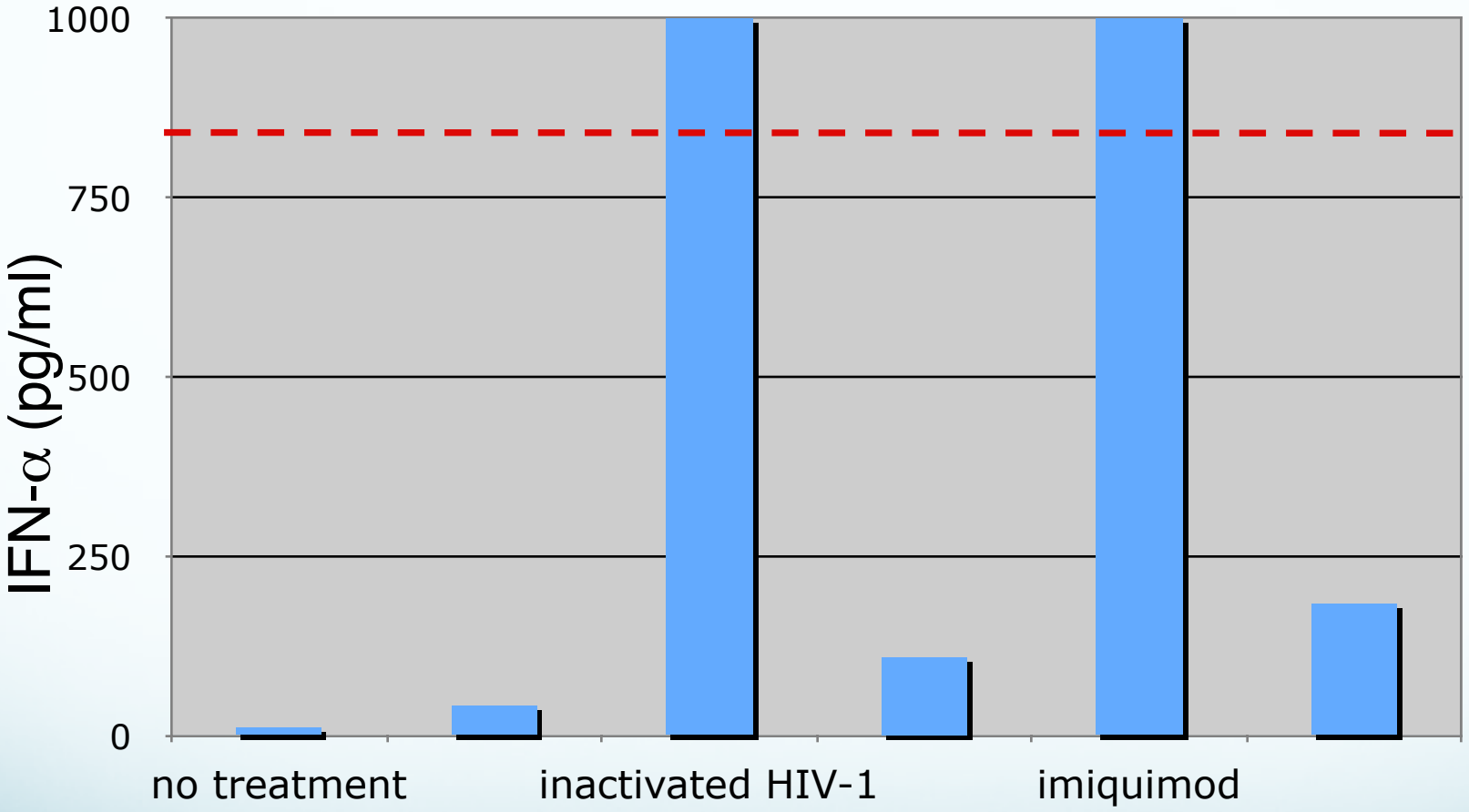
Viruses can interfere with immune responses

Many viruses use DC to facilitate spread

Interactions of Viral or Self nucleic Acid with plasmacytoid DCs



Level of IFN-α Secreted by pDC Exposed to Viruses and TLR7 Agonist



Dendritic cells treated with chloroquine modulate experimental autoimmune encephalomyelitis

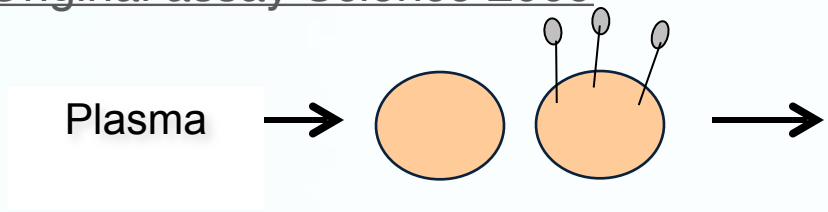
Rodolfo Thomé[1], Luidy Kazuo Issayama[1], Rosaria DiGangi, Andre Luis Bombeiro, Thiago Alves da Costa, Isadora Tassinari Ferreira, Alexandre Leite Rodrigues de Oliveira and Liana Verinaud

Chloroquine (CQ), an antimalarial drug, has been shown to modulate the immune system and reduce the severity of experimental autoimmune encephalomyelitis (EAE). The mechanisms of disease suppression are dependent on regulatory T cell induction, although Tregs-independent mechanisms exist. We aimed to evaluate whether CQ is capable to modulate bone marrow-derived dendritic cells (DCs) both phenotypically and functionally as well as whether transfer of CQ-modulated DCs reduces EAE course. Our results show that CQ-treated DCs presented altered ultrastructure morphology and lower expression of molecules involved in antigen presentation. Consequently, T cell proliferation was diminished in coculture experiments. When transferred into EAE mice, DC-CQ was able to reduce the clinical manifestation of the disease through the modulation of the immune response against neuroantigens. The data presented herein indicate that chloroquine-mediated modulation of the immune system is achieved by a direct effect on DCs and that DC-CQ adoptive transfer may be a promising approach for avoiding drug toxicity.

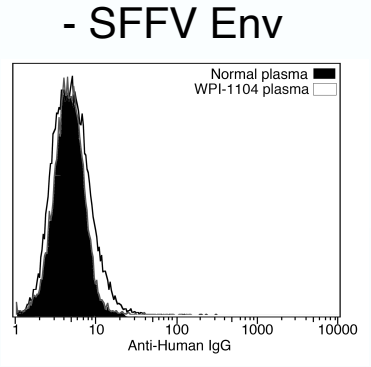
Immunology and Cell Biology (2014) **92**, 124–132; doi:10.1038/icb.2013.73; published online 12 November 2013

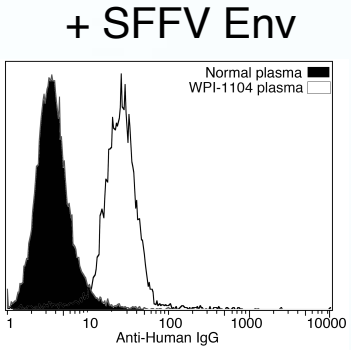
The biomarker for patient population in our studies is the antibody to SFFV–ENV-SU

Original assay Science 2009



FACS analysis of Murine Cell Line
+/- SFFV Env





Plasma from CFS patients block binding of SFFV Env rat mAb to the B cell line expressing SFFV Env, demonstrating specificity

An ANTIBODY POSITVE RESULT DOES NOT NECESSARILY SHOW THE PRESENCE OF A REPLCIATION COMEPTENT RETROVIRUS

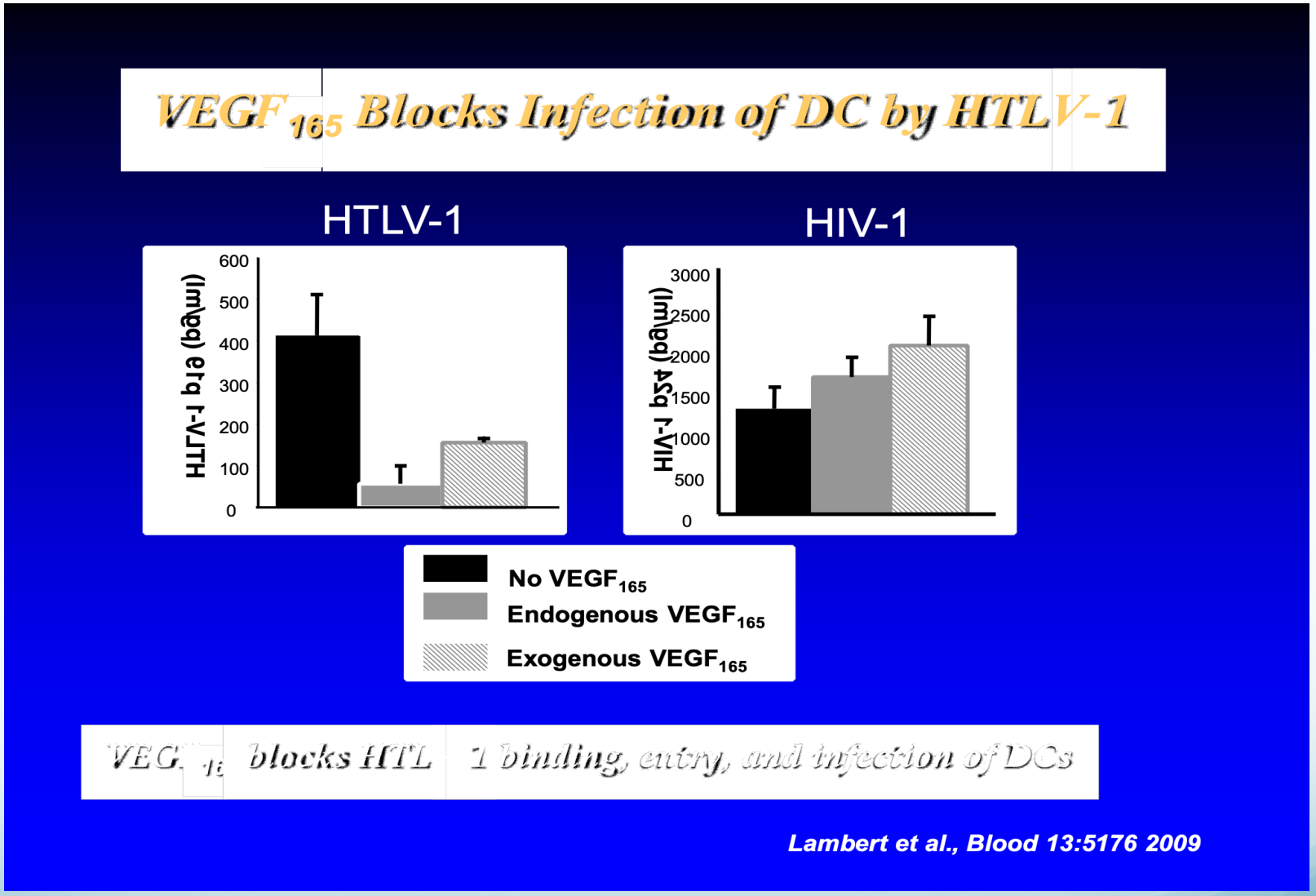
What Could be the basis of SFFV ENV Reactivity in Man ?

HTLV/STLVs SU Contains a VEGF exon 8-like Motif

	** *
$VEGF_{165}$ exon 8	C D **K P** R **R**
HTLV-1 SU (90-94)	K **K P** N **R**

- HTLV-1 SU has a region homologous to $VEGF_{165}$ exon 8
- Consensus motif (KPxR) found in 99% (308/311) of HTLV and STLV SU
- This region contains the 3 residues in $VEGF_{165}$ that directly interact with NRP-1
- Arg 94 residue of SU- critical for infection (Delamarre, J. Virol, 1997)

What does VEGF mimicry mean with regard to drug repurposing?



ARVs provide therapeutic benefit in some patients with autoimmune, Neuroimmune Disease and Cancer

Beneficial Effects could be against:

- An exogenous Replication Competent Retroviruses
- An expressed endogenous virus in an immune compromised individual
- A defective virus expressing only viral proteins
- Aberrantly expressed cellular RNA including miRNA (regulatory)

The Latest “ome” : Metabolome studies reveal new uses for old drugs

OPEN ACCESS Freely available online

PLOS ONE

Antipurinergic Therapy Corrects the Autism-Like Features in the Poly(IC) Mouse Model

Robert K. Naviaux[1,2,3,4*], Zarazuela Zolkipli[1,5], Lin Wang[1,2], Tomohiro Nakayama[1,5], Jane C. Naviaux[1,6], Thuy P. Le[1,3], Michael A. Schuchbauer[6], Mihael Rogac[1,2¤], Qingbo Tang[2], Laura L. Dugan[2], Susan B. Powell[6]

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Suramin

Conclusion

Drug repurposing holds tremendous promise of *IMMEDIATE* new therapies for patients with complex chronic diseases including:

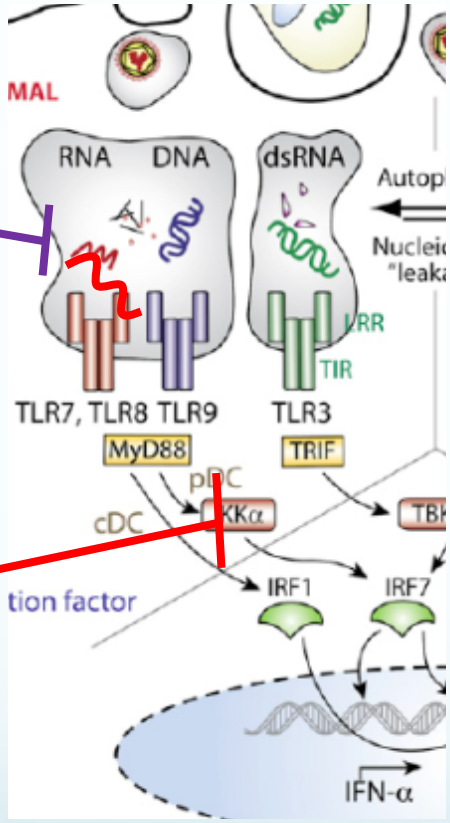
- ME/CFS
- CLD
- ASD
- MS
- PD
- ALS
- AD

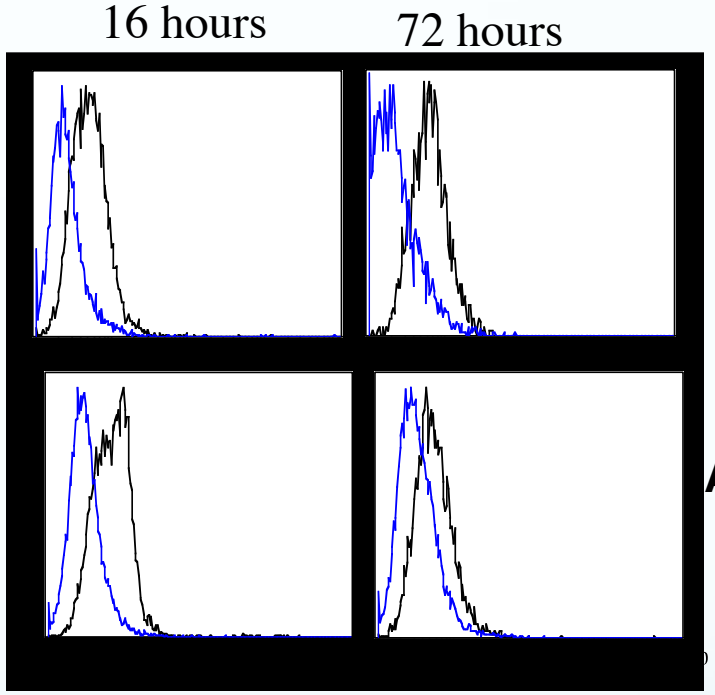
BACKUP SLIDES

Is IFN-α Production in pDC Exposed to HTLV-1 Blocked by Competition for TLR Signaling?

HTLV-1 RNA?

HTLV-1 protein?





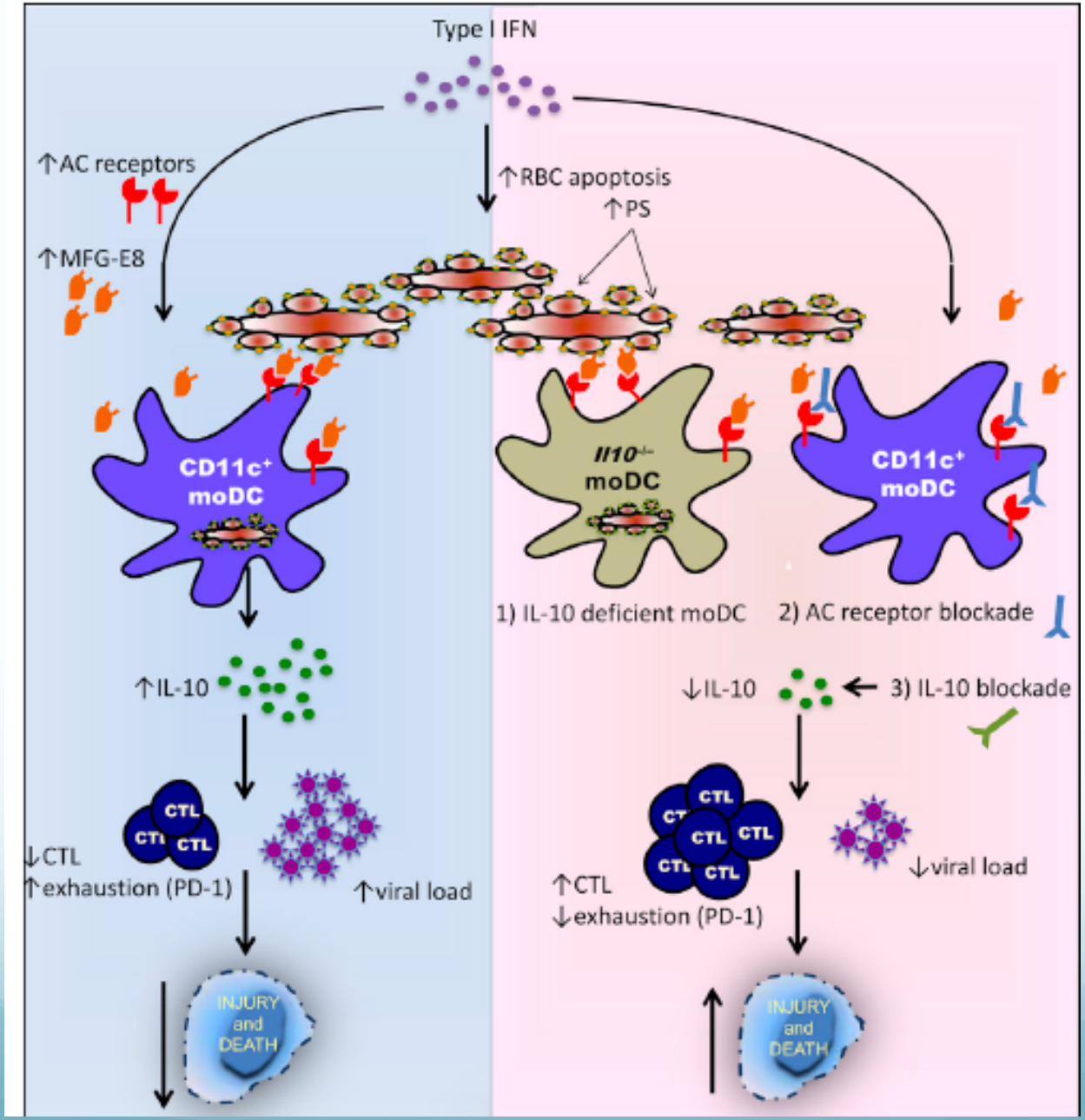
Exposed to AT-2 HIV

Exposed to AT-2 HIV and HTLV-1

Intracellular IFN-α

Appears block to IFN-α production is subsequent to TLR signaling

Elevation of type 1 IFN leads to apoptosis of RBC, exhaustion of CTL and poor Virus control



GASTROENTEROLOGY 2012;143:1586–1596

Altered Functions of Plasmacytoid Dendritic Cells and Reduced Cytolytic Activity of Natural Killer Cells in Patients With Chronic HBV Infection

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Microbiota effects on immunity

Review

Trends in Immunology September 2012, Vol. 33, No. 9