Dysregulation of Innate Immunity in Aging

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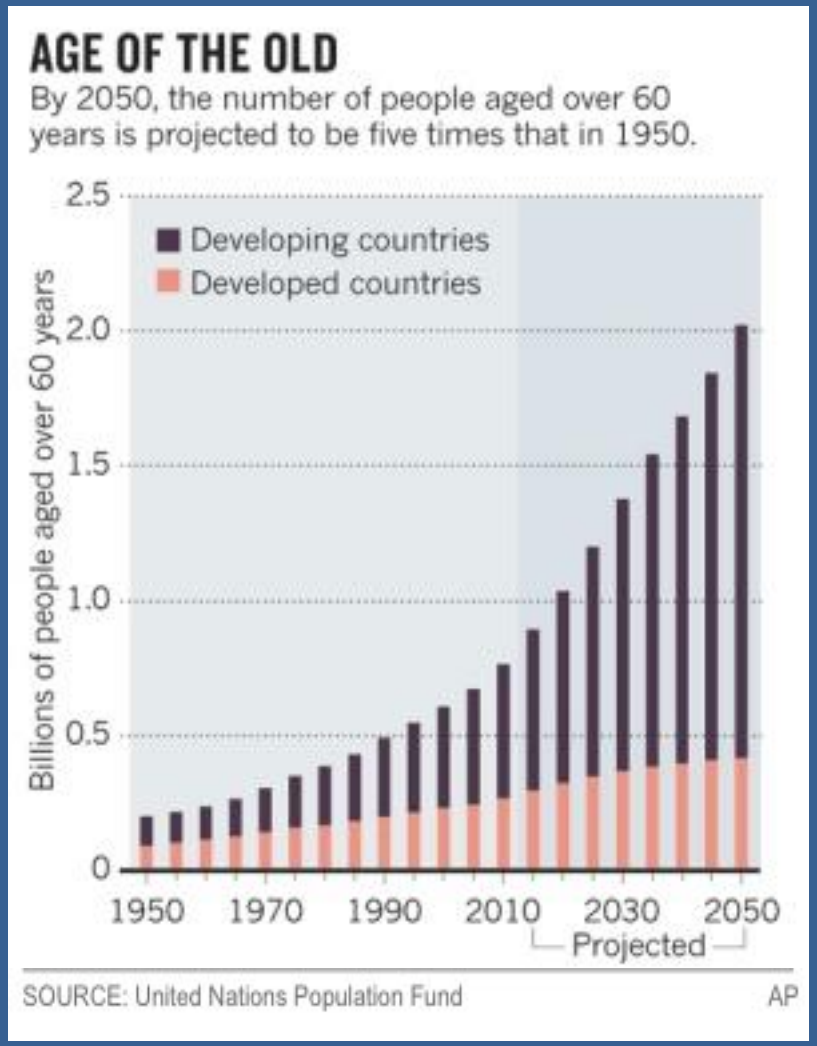
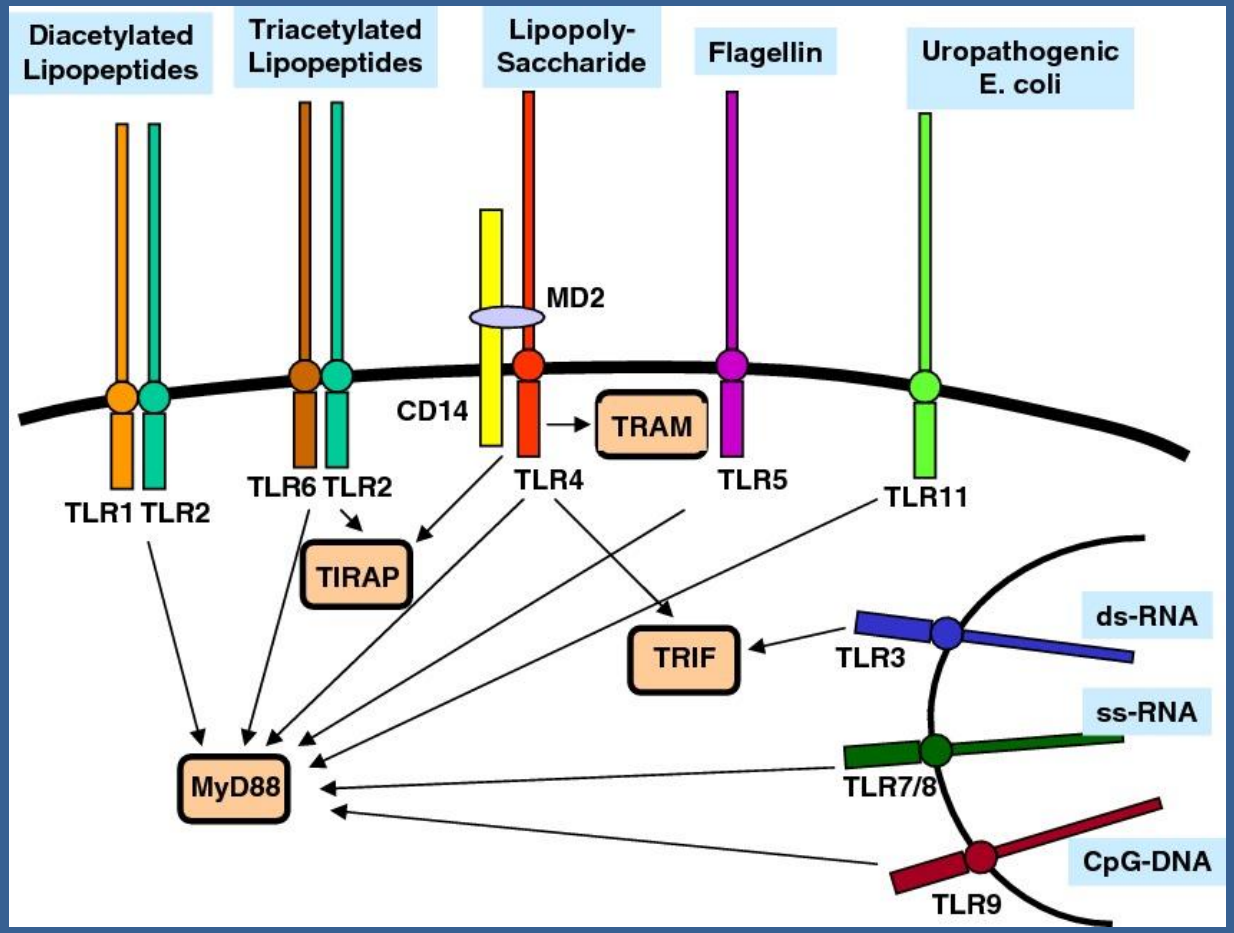
The Geriatric Demographic Imperative: Population over age 65 (millions)



- **The number of people > age 60 will exceed 2 billion by 2050**
- **Individuals over age 65 who currently comprise about 12% of the US population account for over 35% of visits to general internists, 34% of prescription drug use, 50% of hospital stays, and 90% of nursing home residents (CDC, 2005).**

Toll-like receptors (TLRs) and agonists: repeated patterns of pathogens

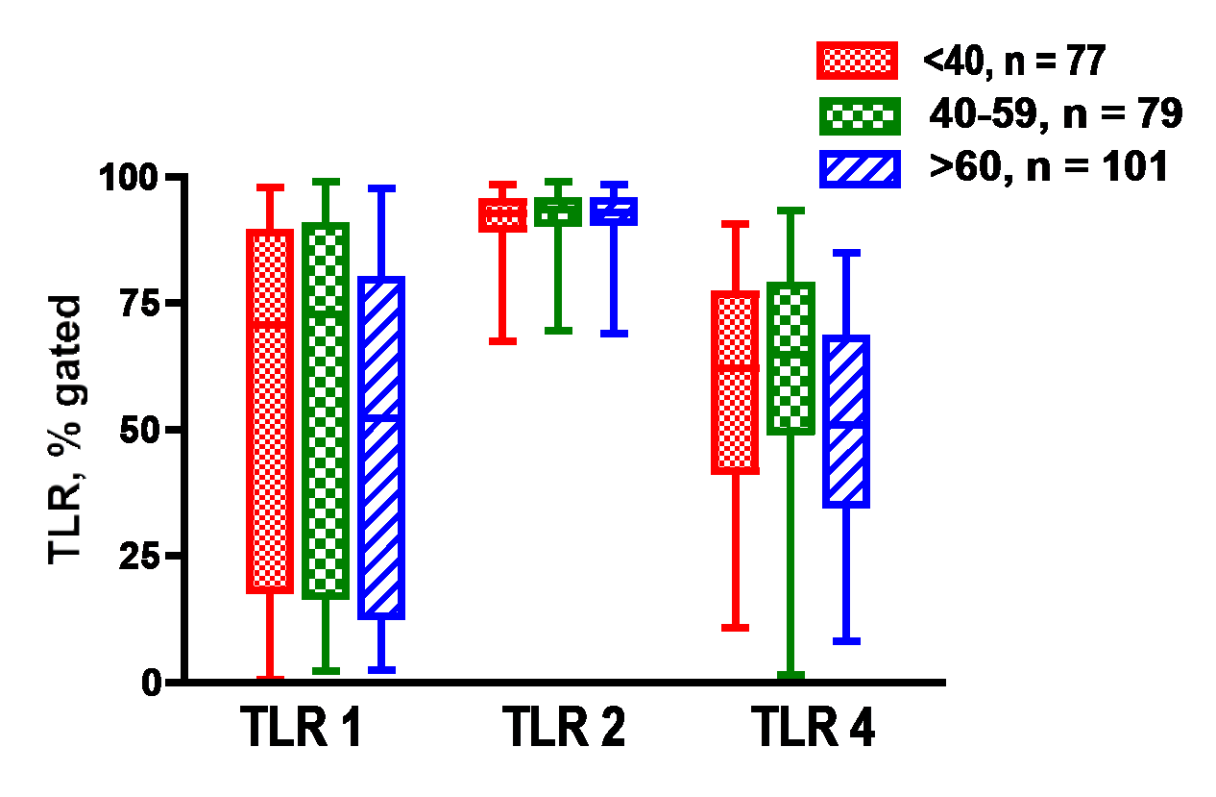


Terjung et al, Clinic Rev Allerg Immunol (2009) 36:40–51

TLR ligand binding activates MyD88/TRIF which:

- Activates NFκB
- Activates interferon-regulatory factors (IRFs)
- Activates other transcription factors
- Leads to production of cytokines (e.g. TNFα, IFN)

TLR expression on monocytes by age group

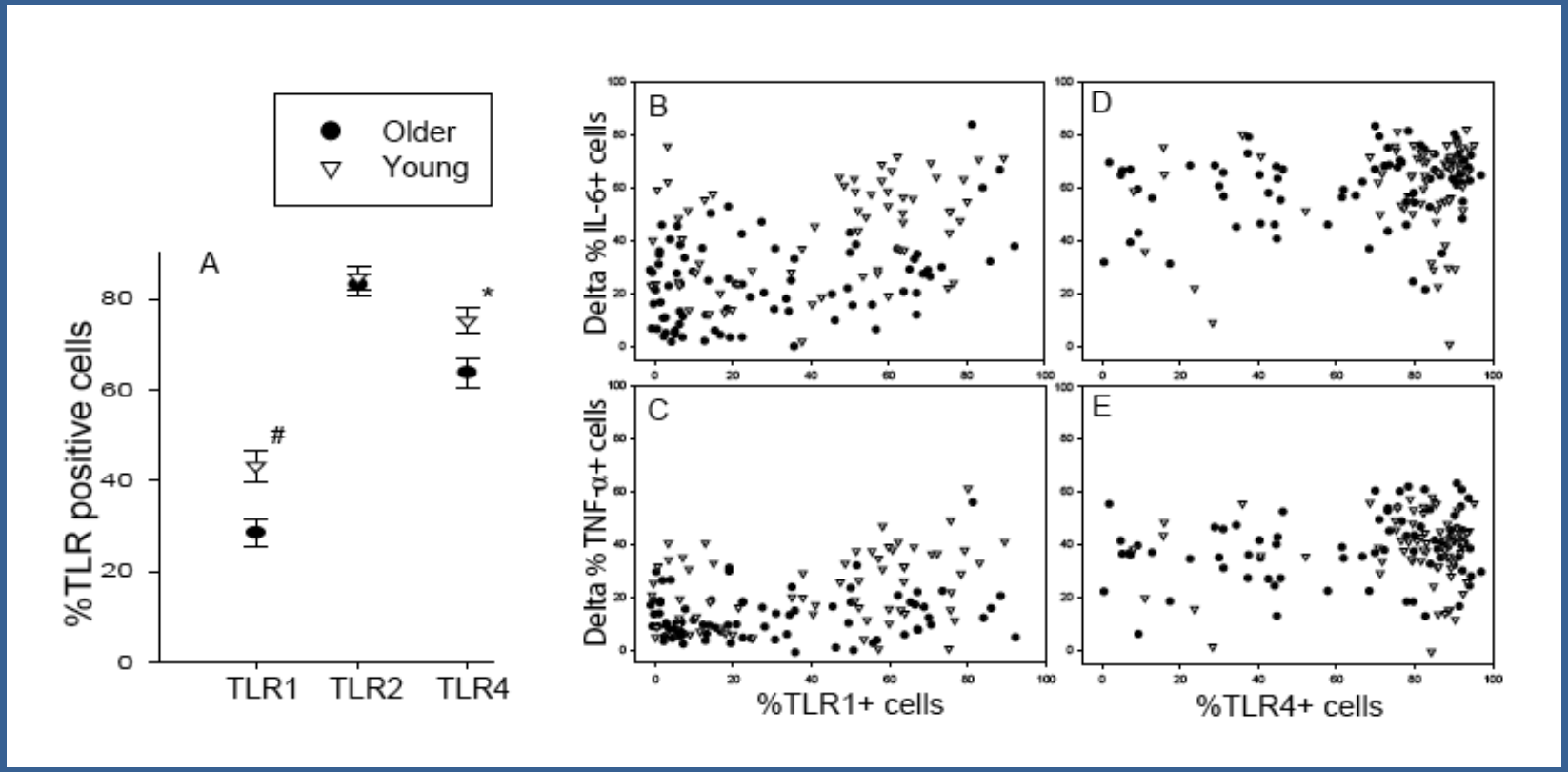


Data shown is the median percentage of gated cells that are positive for the indicated marker. Range (75th and 25th %). p-value is based on the mixed effects model.

	<40 vs >60	40-59 vs >60
TLR 1	P=0.0018	P=0.0012
TLR 2	NS	NS
TLR 4	P=0.0002	P<0.0001

van Duin, et al. 2007. *J. Immunol.* 178: 970-975.

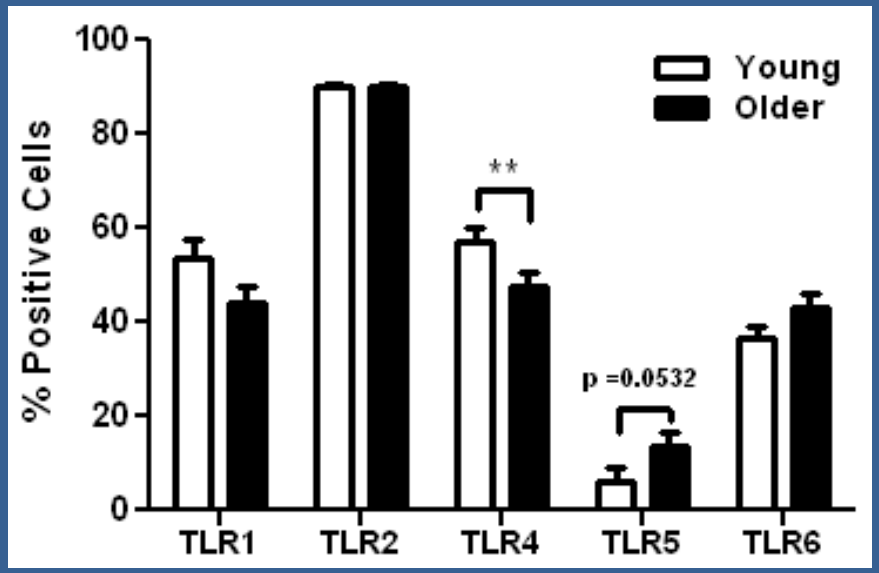
Age-associated Decrease in TLR1 Surface Expression Correlates with Decreased Cytokine Production



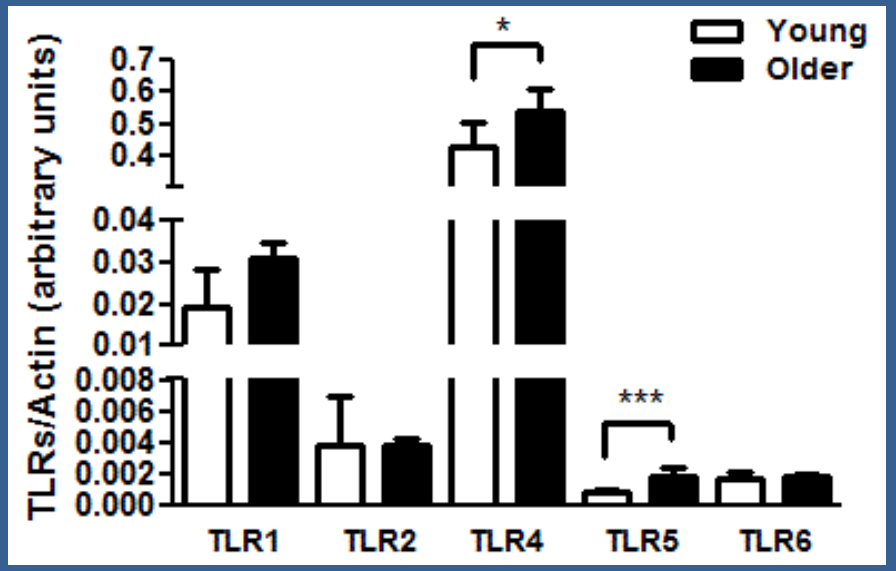
A. CD11c+ monocytes positive for TLR shown, n = 66 young, n = 80 older. B-E. Correlation of TLR + monocytes with cytokine production (IL-6 $p<0.0001$; TNF $p=0.0003$). Age-related decrease in costimulation markers CD80/86 correlated with reduced response to influenza vaccination.

van Duin, D, Thomas, V, Mohanty, S, **Montgomery, RR**, Ginter, S, Fikrig, E, Allore, HG, Medzhitov, R, and Shaw, AC 2007 *J. Immunol.* 178:970-975.

Age-associated alterations in TLR expression in human monocytes



Percent positive monocytes for surface expression of TLR1, TLR2, TLR4 (older n=118, young n=79), TLR5 (older n=38, young n=47) and TLR6 (older n=90, young n=69). Means ± SEM (** $p < 0.01$).



Median with interquartile range of mRNA expression levels of TLRs in purified monocytes (n=23 young, n=26 older); * $p < 0.05$ and ***$p < 0.001$.

Elevated levels of TLR5 leads to
- increased IL-8 production and through
- Increased levels of p38 and ERK MAPK signaling pathway

Qian, et al. *2012. Aging Cell* ***11:***104-110.

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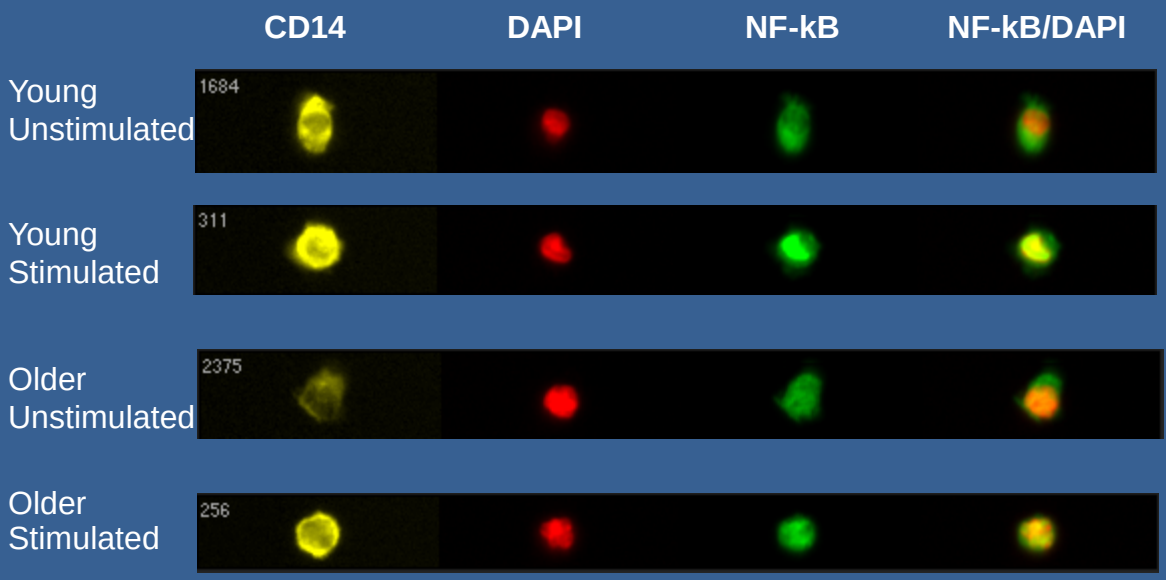
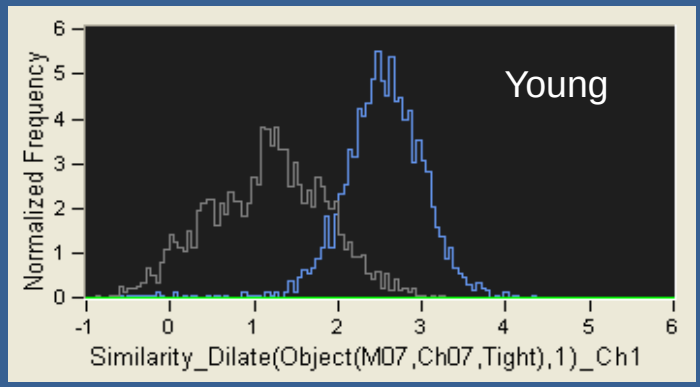
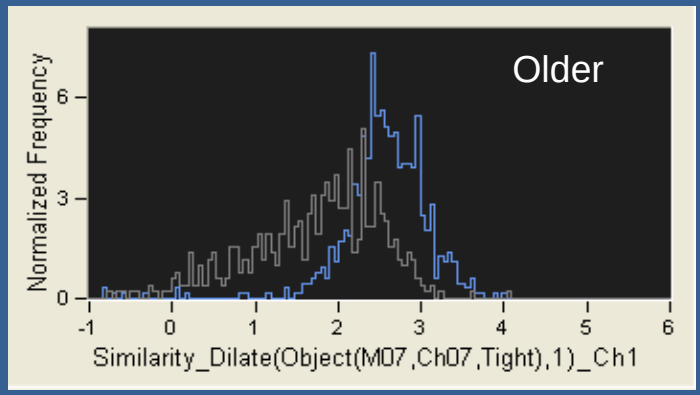
Studies of mechanisms underlying dysregulation in aging

ImageStream: Imaging Flow Cytometer

Quantitative detection of nuclear translocation of transcription factor NF-κB simultaneously in single cells and populations







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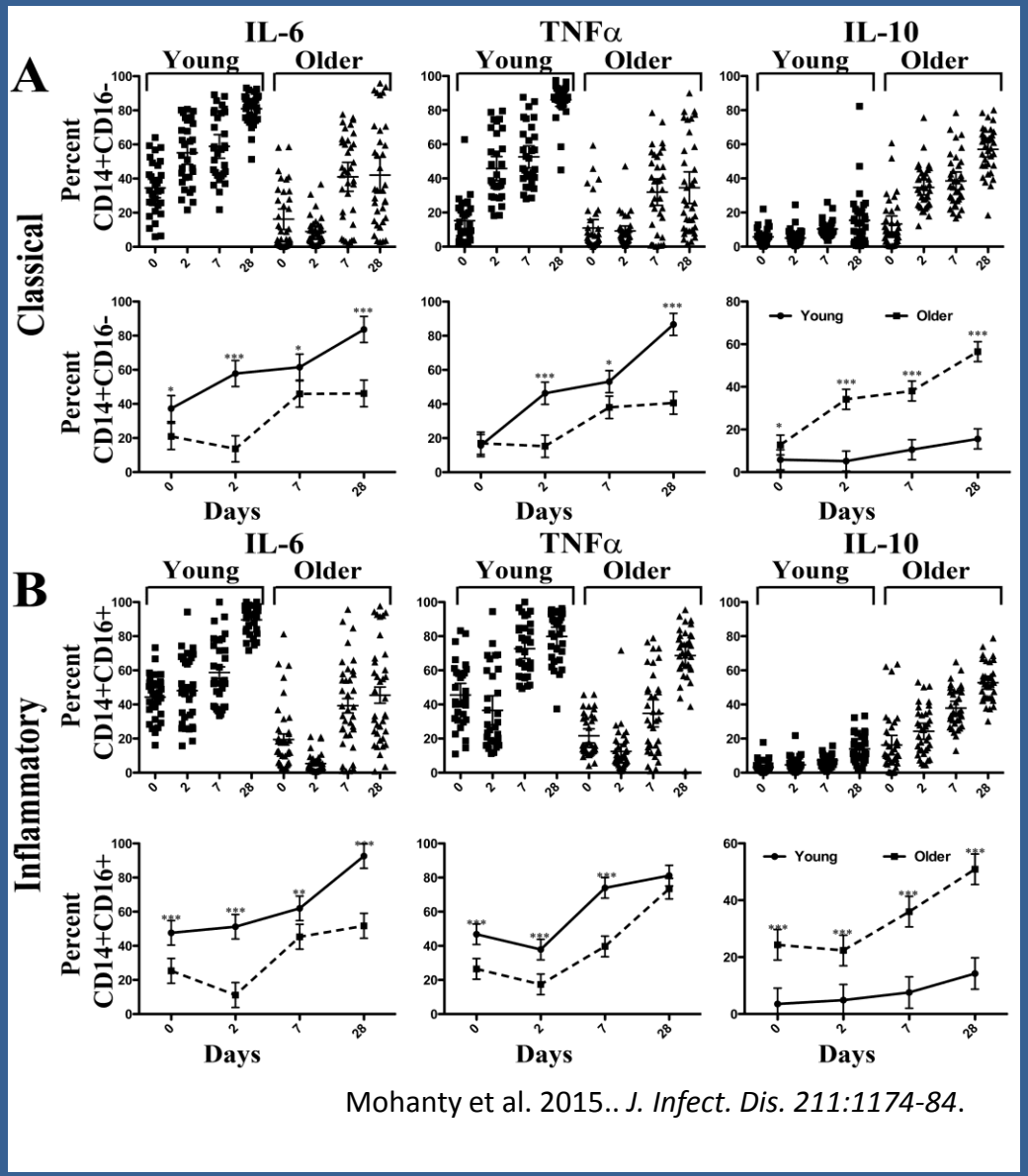
Qian, et al. *2012. Aging Cell* ***11:****104-110.*

Dysregulated IL-10 production in impaired vaccine responses in older adults

- PBMCs collected on Day 0, 2, 7, 28 following vaccination
- CD14+ CD16+ inflammatory monocytes were induced following vaccination
- Classical CD14+ monocytes TNF-α and IL-6 production were strongly induced following vaccination
- anti-inflammatory cytokine IL-10 was markedly elevated in monocytes from older subjects
- age-associated elevation in phosphorylated STAT3
- decreased phosphorylation of DUSP1 (serine 359) the negative regulator of IL-10

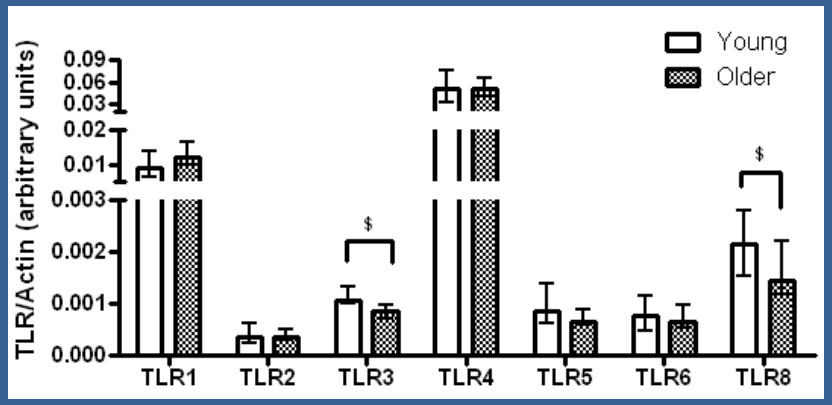


Mohanty et al. 2015.. *J. Infect. Dis. 211:1174-84*.

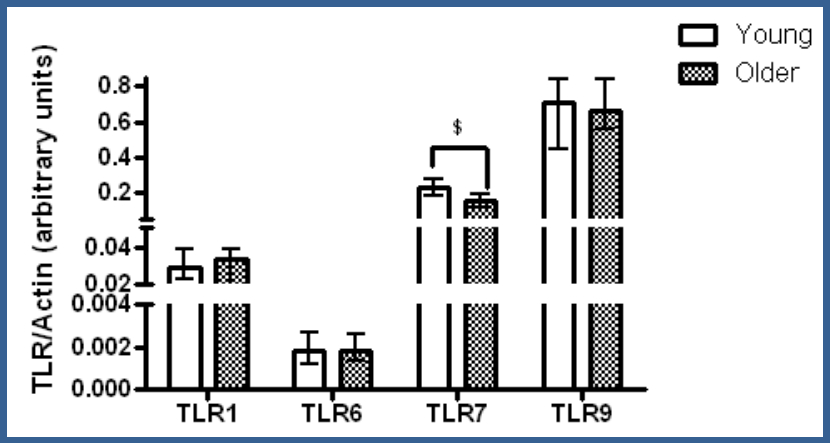
Age-associated Alteration in TLRs in DCs

Reduced TLR 3, TLR7, TLR 8

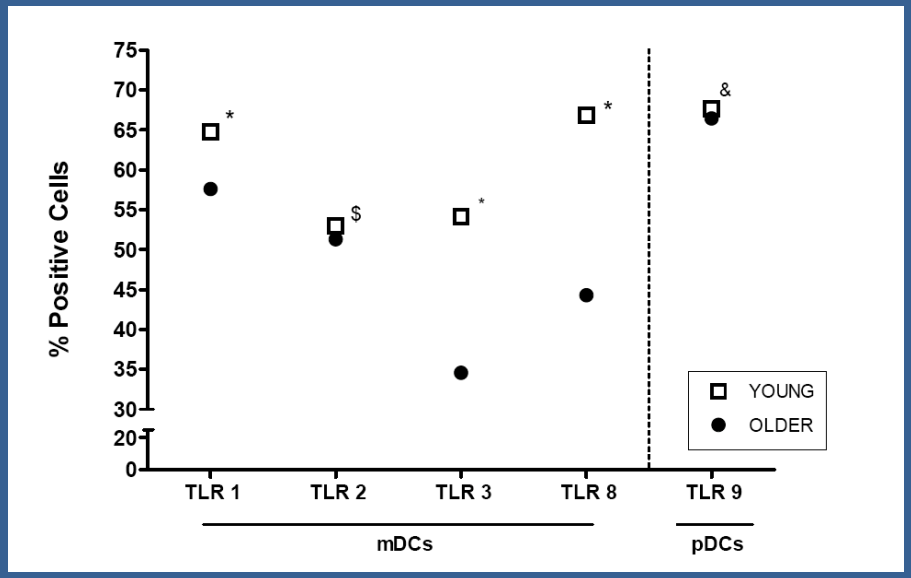
Q-PCR mDC



Q-PCR pDC



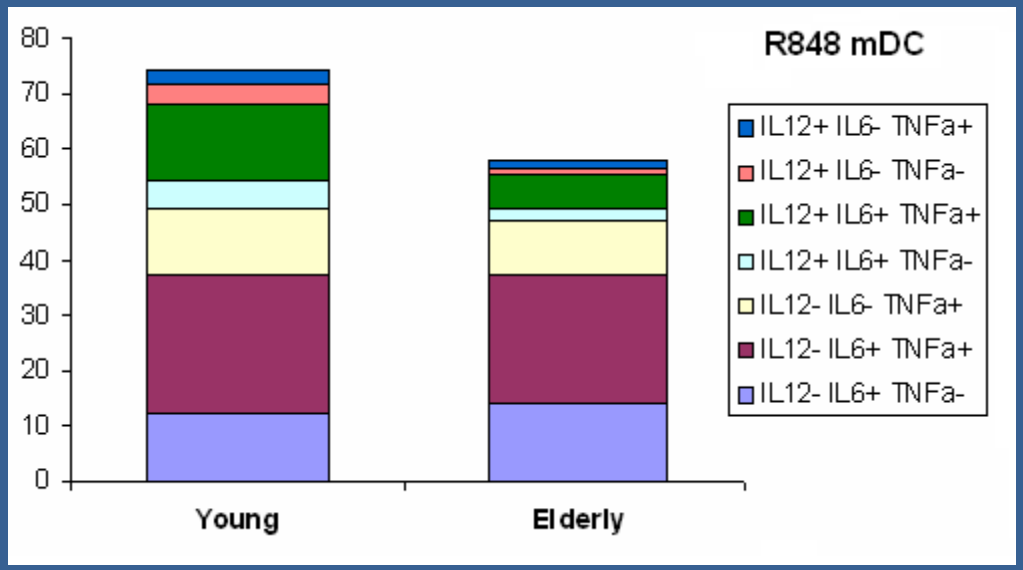
FACS: mDC & pDC



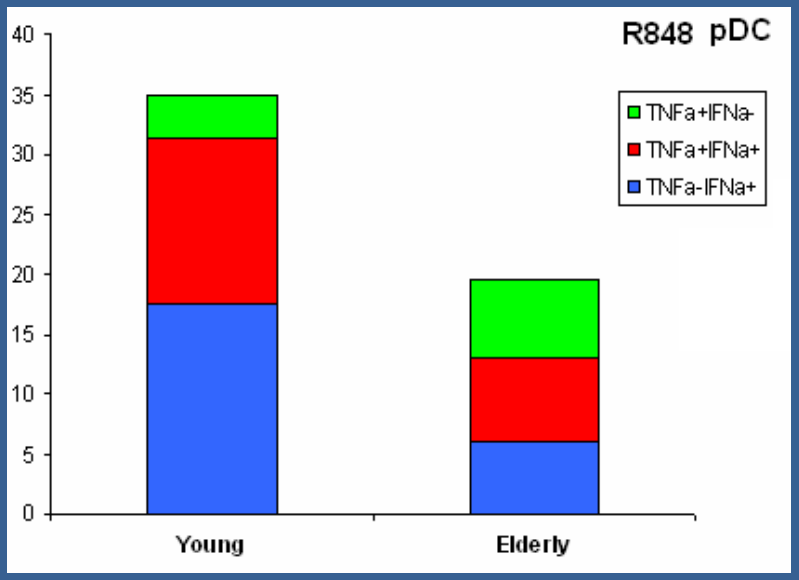
Panda, A., Qian, F., Mohanty, S., van Duin, D., Newman, F.K., Zhang, L., Chen, S., Towle, V., Belshe, R.B., Fikrig, E., Allore, H.G., Montgomery, R.R., and Shaw, A.C. 2010. *J Immunol* 184:2518-2527.

Boolean Gating of DC Populations: Effect of R848 stimulation (TLR 7/8)

mDC



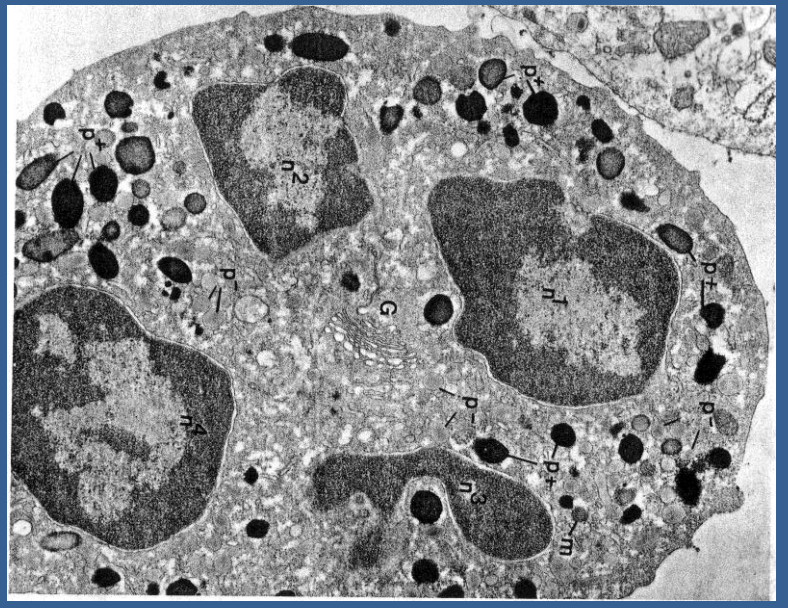
pDC

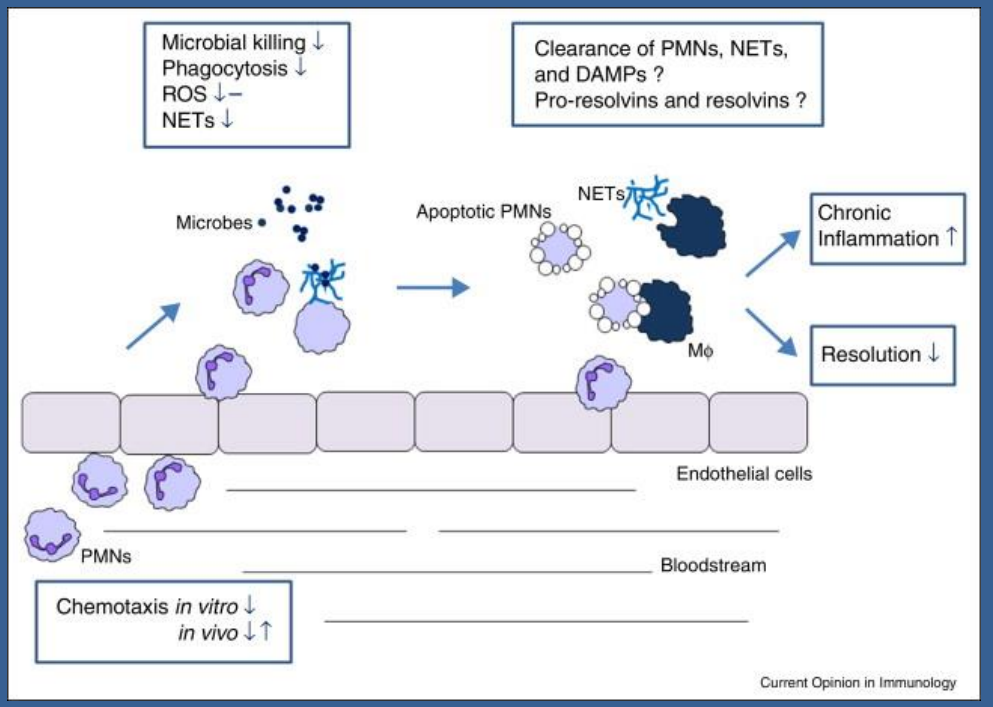


In DCs from older donors:
Lower levels of TLRs (Q-PCR & Flow cytometry)
Lower induction of triple positive effector cells

Neutrophils in Aging

- Cell number equal in aging
- Migration speed equal
- Chemotaxis impaired, i.e. reduced ability to traffic to sites of infection, also defective neutrophil egress from inflamed tissue
- Reduced soluble mediator release (O_2-, proteases, cytokines)
- Reduced phagocytosis
- Reduced pathogen killing
- Fewer Neutrophil extracellular traps (NETs), or scaffolds of extruded chromatin, antimicrobial peptides and proteases (elastase, MPO)
- dysregulated signal transduction receptors through inappropriate retention in lipid rafts (Fulop, 2004)





Tseng and Liu, Current Opinion in Immunology, Volume 29, 2014, 43 - 48

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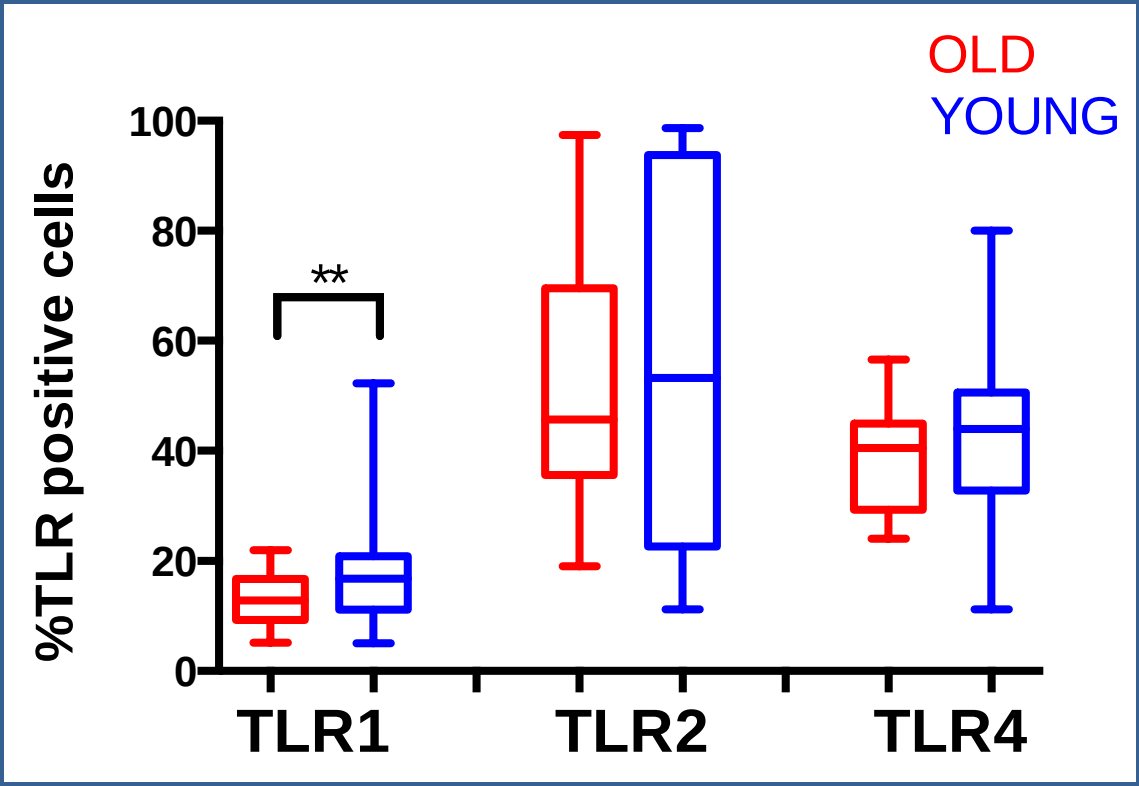
Age associated decrease in TLR1 expression levels on PMN



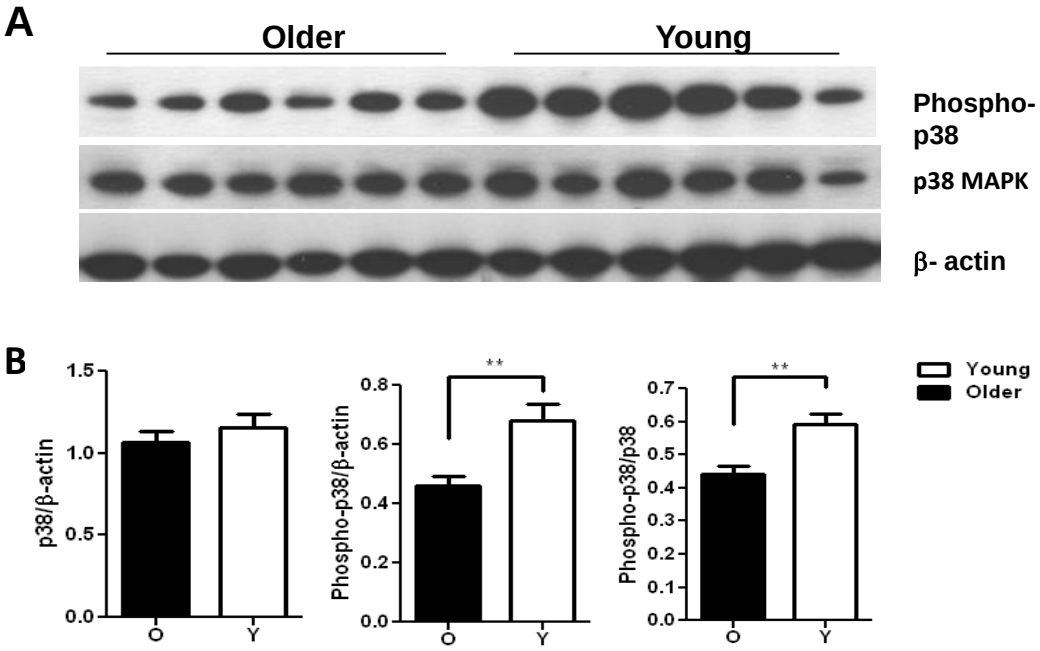
Age-associated alterations in toll-like receptor (TLR) expression in human neutrophils. Whole blood of younger (n=31) and older (n =22) donors was labeled for flow cytometry with lineage markers and TLRs at 4˚C for 30 min following RBC lysis. Labeling was detected by LSR II. Data shown are % positive neutrophils for TLR surface expression. Values indicate the medians and 25th and 75th percentiles in young and older adults. Asterisks indicate statistical significance between young and older cohort (unadjusted t-test accounting for unequal variances, ** $p < 0.02$).

Qian, et al . 2014.. *Aging* 6: 131-139.

Age-associated reduction in TLR1-mediated activation of PMN

DECREASED IN AGING:

- TLR-1 induced increase in integrins CD11b, CD18
- TLR-1 induced production of IL-8
- TLR-1 mediated rescue of apoptosis
- TLR-1 mediated phosphorylation of p38 MAPK



PMN were stimulated with the TLR1/2 ligand Pam3CSK4 for 15 min. (A) Immunoblot of p38 phosphorylation in a representative result (n=6/group). (B) Densitometry of immunoblot of p38 and phospho-p38 in PMN (n =21/group). Means ± SEM of the ratio of total p38 and phospho-p38 to β-actin, and of phospho-p38 to total p38; (** $p < 0.01$).

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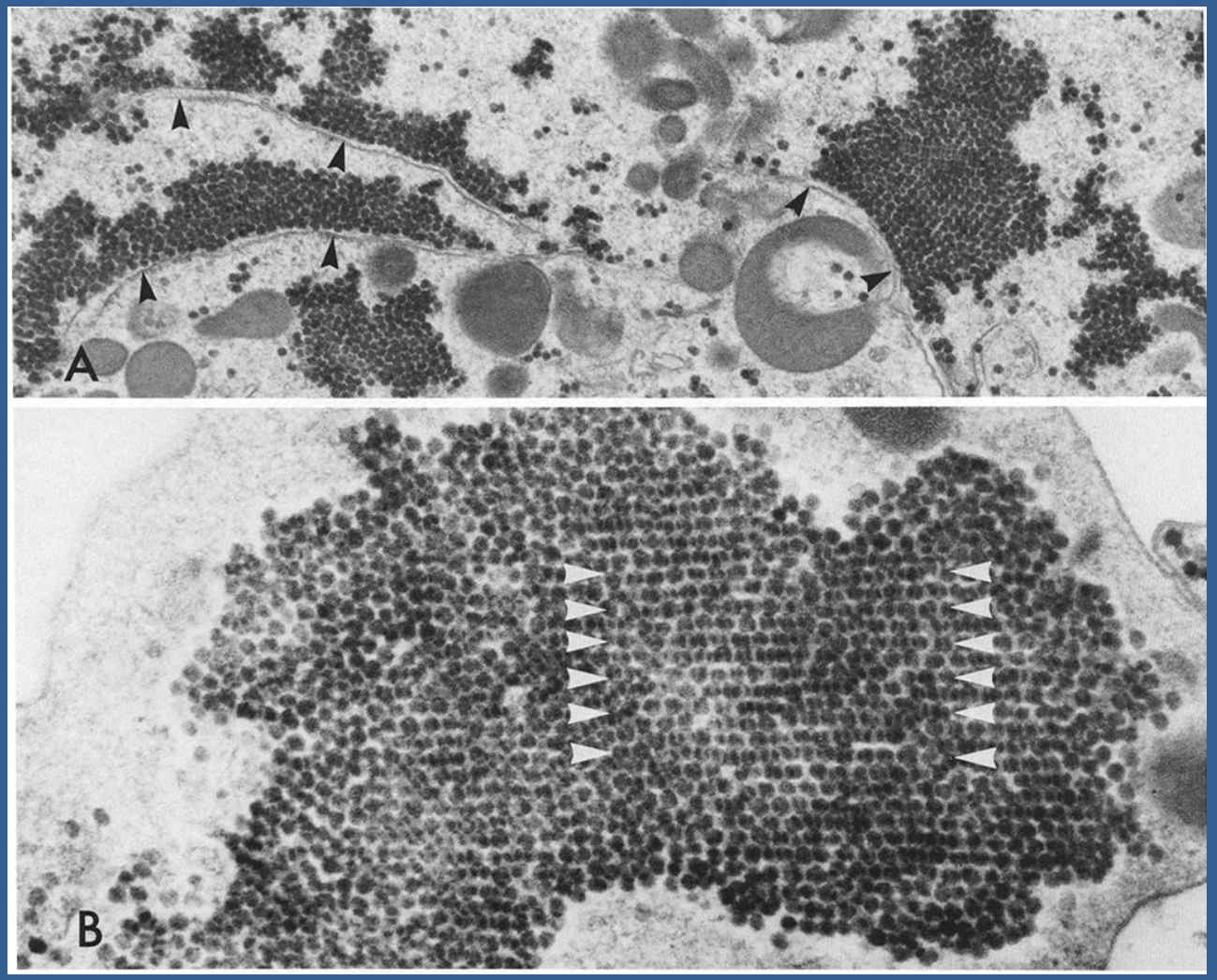
Qian, F., X. Guo, X. Wang, X. Yuan, S. Chen, S. E. Malawista, L. K. Bockenstedt, H. G. Allore, and R. R. Montgomery. 2014.. *Aging* 6: 131-139.

PMN accumulate glycogen during inflammation

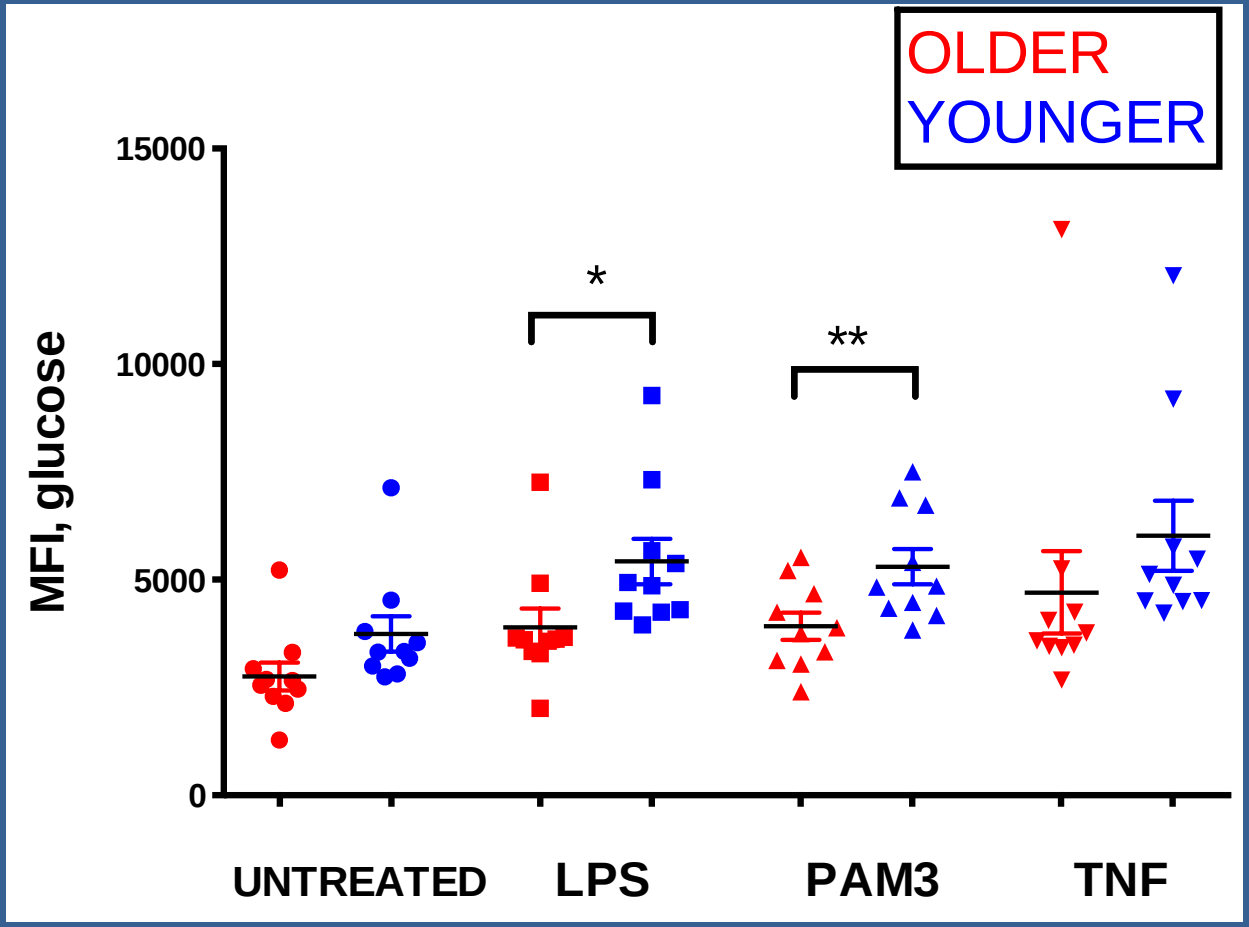


Electron micrograph of guinea pig neutrophils elicited with LPS shows paracrystalline arrays of glycogen granules.

Robinson, Karnovsky, & Karnovsky, J. Cell. Biol., 1982.

Effect of Aging on Bioenergetics of PMN

Bioenergetics underlie mechanisms relevant to critical PMN actions for anti-microbial activities, as reservoir for bacteria or viruses, or activation of adaptive responses.



Reduced accumulation of glucose in PMN in aging. Levels of fluorescent glucose detected by flow cytometry after 2 hr ± stimulation of PMN from younger and older donors. Stimulation > untreated for both age groups; Young > Old (* p= 0.04, ** p=0.02; n=10/group).

Qian, F., X. Guo, X. Wang, X. Yuan, S. Chen, S. E. Malawista, L. K. Bockenstedt, H. G. Allore, and R. R. Montgomery. 2014.. *Aging* 6: 131-139.

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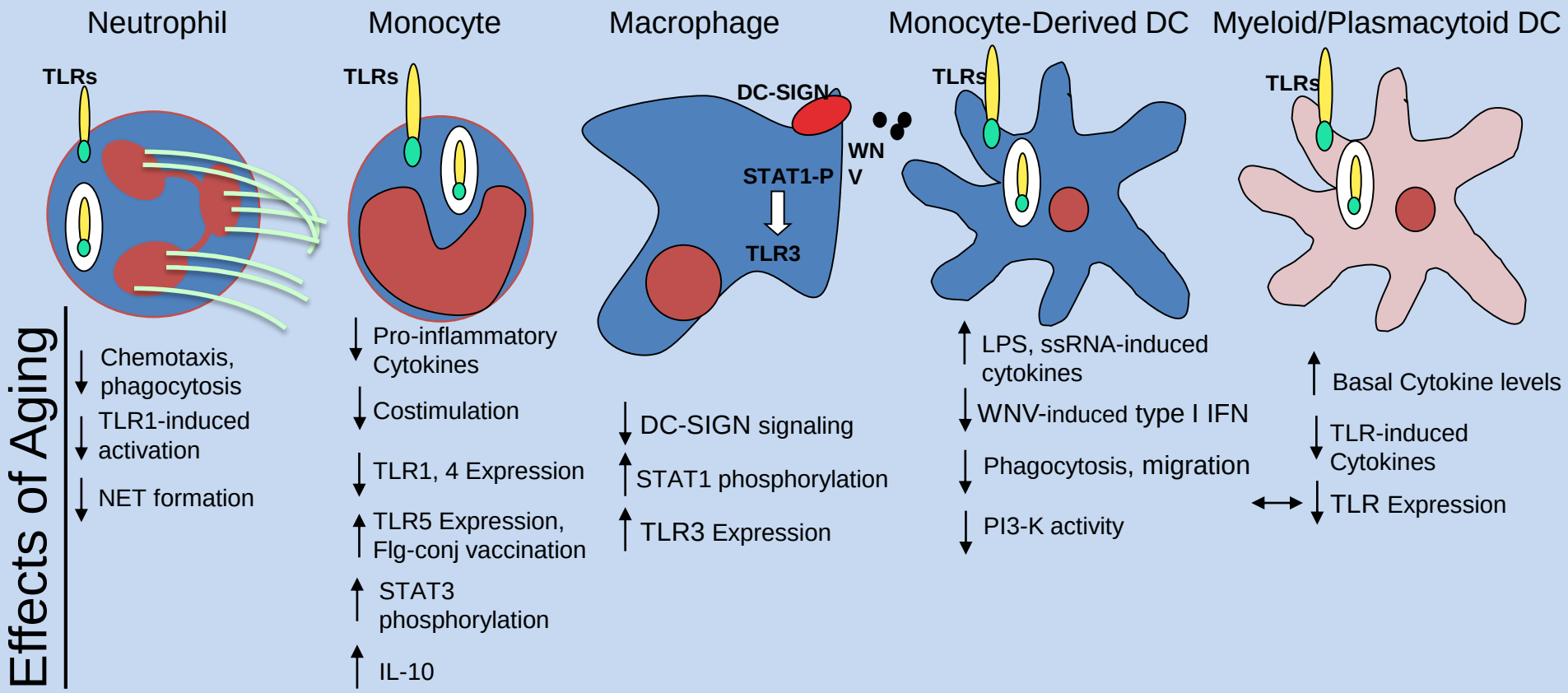
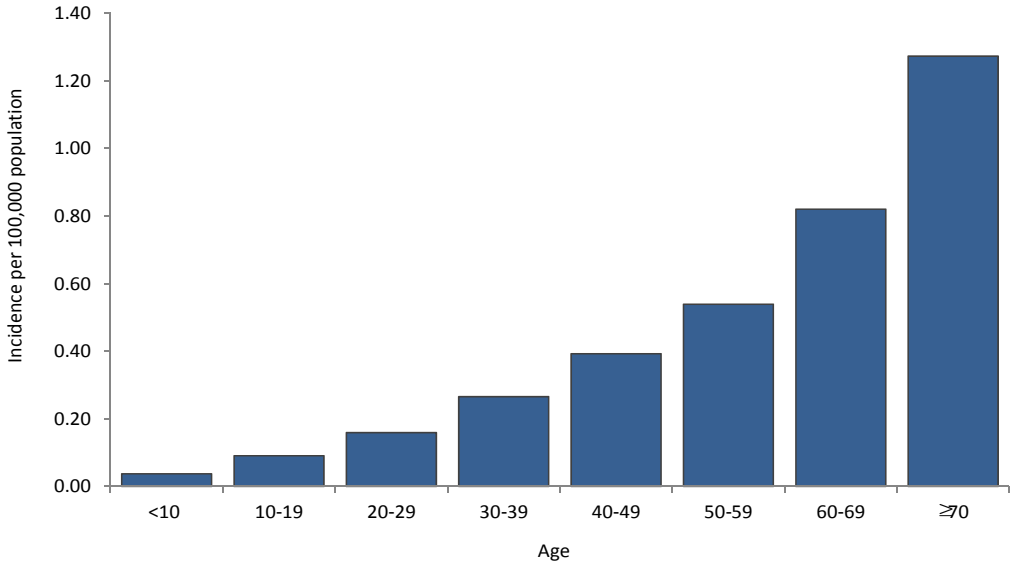
Effects of Aging on Innate immune Cells



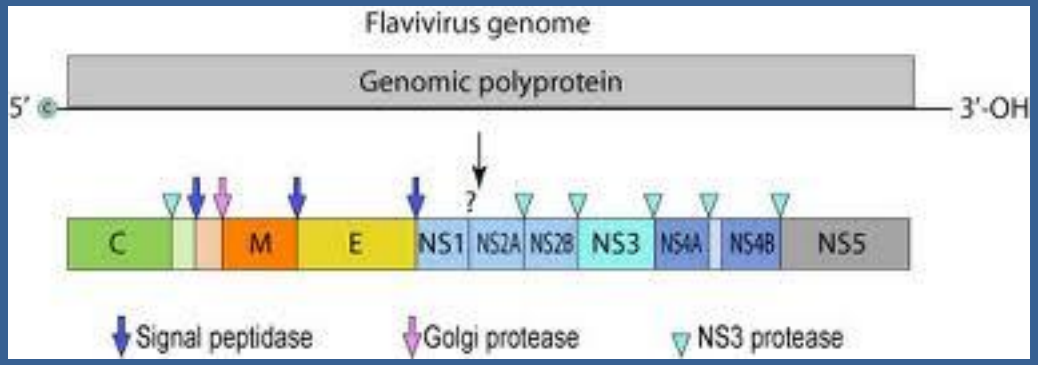
Incidence of WNV neuroinvasive disease by age

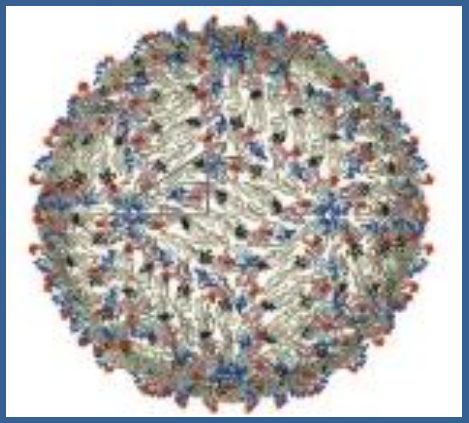
Average annual incidence of West Nile virus neuroinvasive disease reported to CDC by age group, 1999-2014



Source: ArboNET, Arboviral Diseases Branch, Centers for Disease Control and Prevention

Flavivirus Genome





Crystal structure of WNV
Kanai, R, et al., JVI, 2006.

- Linear, positive sense single-stranded RNA genome, 9-12 kb
- Primarily spread through arthropod vectors
- Major diseases caused by the *Flaviviridae* family include:

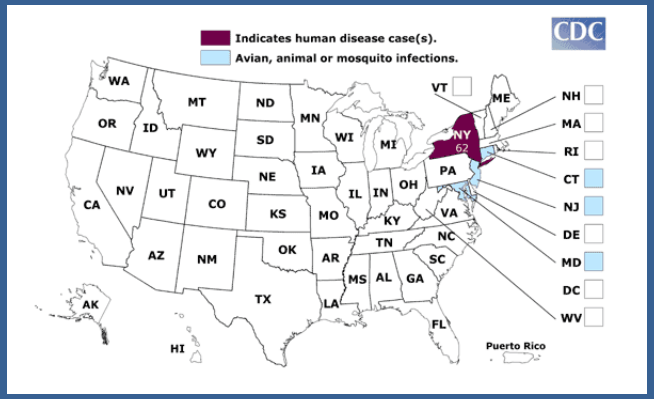
West Nile encephalitis
Japanese Encephalitis
Kyasanur Forest disease
Murray Valley encephalitis
St. Louis encephalitis

Tick-borne encephalitis
Yellow fever (Best vaccine ever ?)
Hepatitis C virus (170 million pts)
Dengue fever (400 million pts/year)

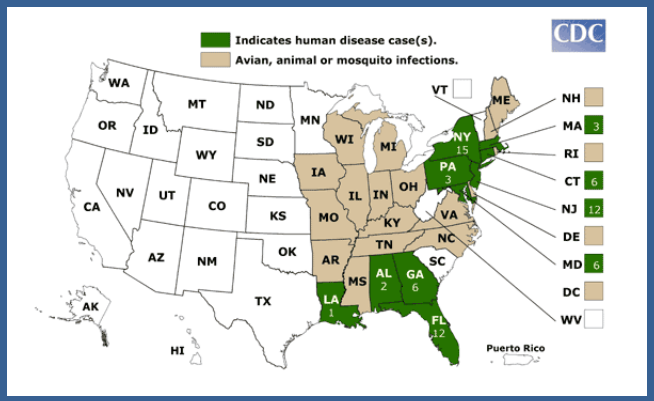
Emergence of West Nile virus

- Positive strand RNA virus in the family *Flaviviridae* containing Dengue, Yellow Fever, and Hepatitis C viruses
- WNV emerged in North America in 1999 in New York City
- Transmitted by the *Culex* and *Aedes* mosquito vectors
- WNV has spread throughout the US and into Canada, Mexico, and the Caribbean
- 2005-2009: 12,975 cases including 496 fatalities were reported in the USA; 35% severe neuroinvasive disease (encephalitis)
- Estimated > 3 million people in the US infected resulting in ~780,000 illnesses
- Elderly and immunocompromised individuals are more susceptible
- no FDA-approved treatments available

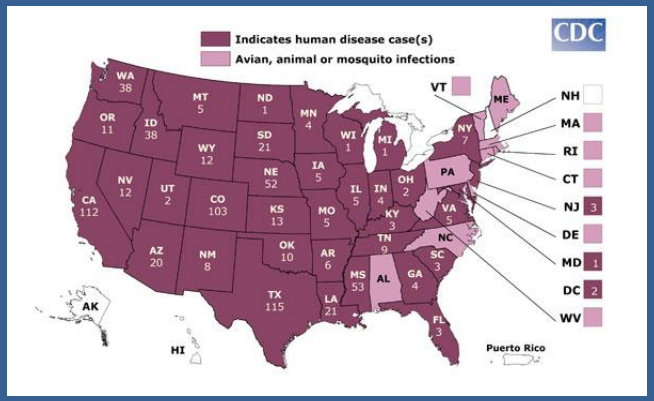
1999



2001



2009



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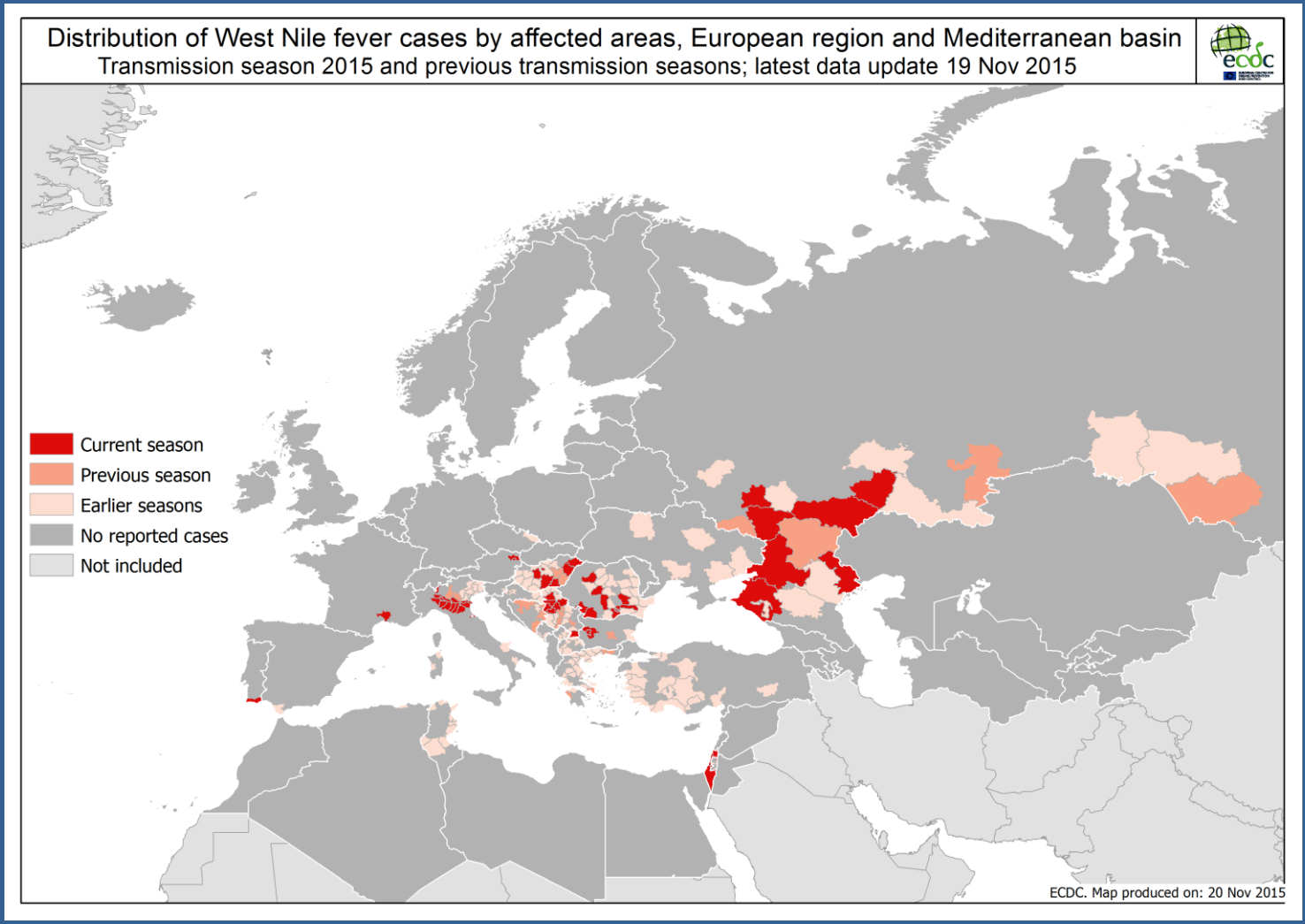
Recent Activity of WNV



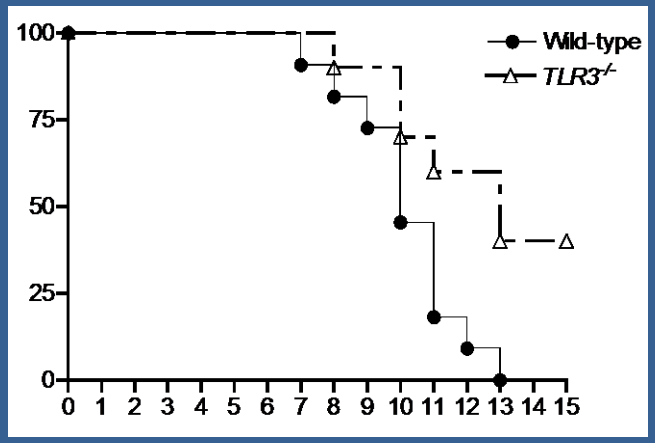
USA:
1,996
cases in
2015

European Centre for Disease Prevention and control reported
As of 19 November 2015, 108 cases of West Nile fever in humans have been reported in the EU Member States

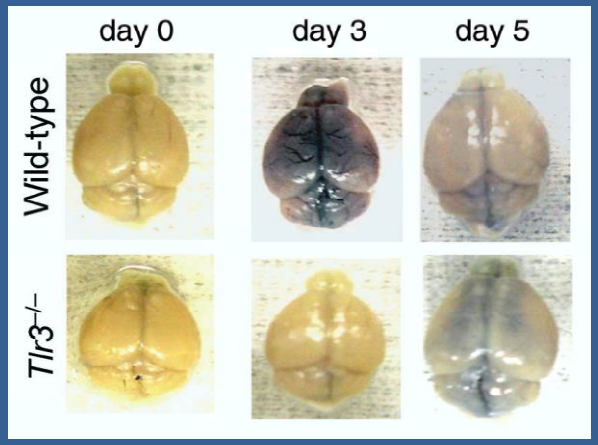
http://ecdc.europa.eu/en/healthtopics/west_nile_fever/west-nile-fever-maps/pages/index.aspx

Role of TLR3 in resistance to WNV

Murine infection model



***TLR3*$^{-/-}$ mice are more resistant to lethal WN virus infection.** Wild-type and *TLR3*$^{-/-}$ mice were infected with LD100 (n=10) doses of WN virus and monitored twice daily for mortality. P value is 0.01. * $P < 0.05$ compared to wild-type mice, n=3.



Brain permeability is increased after WNV infection in wild-type but not TLR3$^{-/-}$ mice. Evans Blue staining of WN virus-infected whole brains (days 0, 3, and 5).

Factors that diminish the permeability of the blood brain barrier (BBB) reduce viral load in the brain and protect from lethal WNV infection.

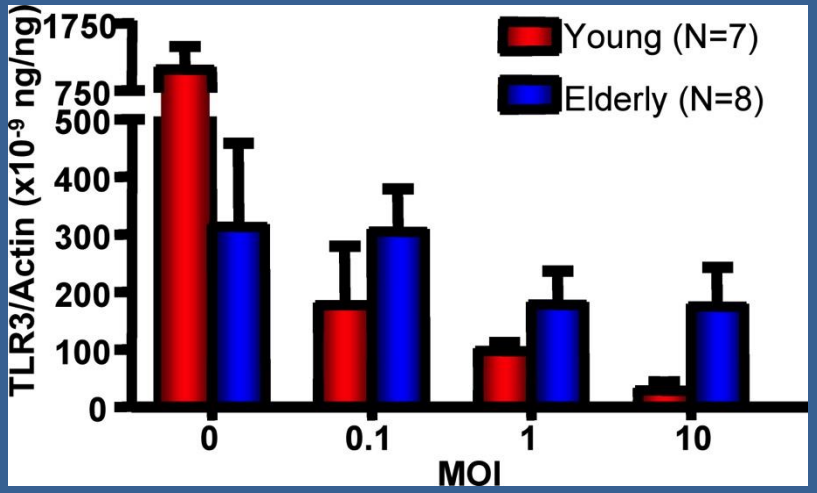
Wang, T., Town, T., Alexoupoulou, L., Anderson, J.F., Fikrig, E., and Flavell, R.A. 2004. *Nat. Med.* 10:1366-1373.

WNV down-regulates TLR3 in macrophages but only from young donors

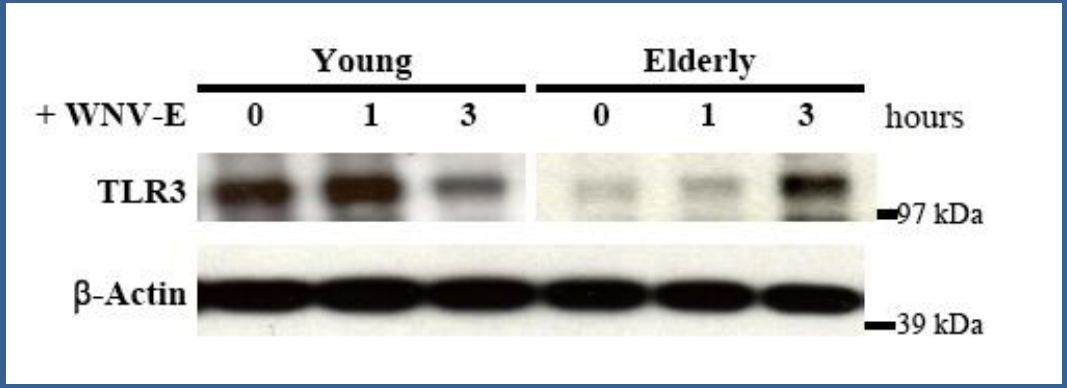
Baseline TLR3 levels lower in elderly

Downregulation of TLR3

- Absent in Elderly
- Dose-dependent
- Fast (1 hr)
- Occurs with WNV & E protein
- Mediated by DC-SIGN
- Requires glycosylated WNV E (does not occur with E154 mutant)



Macrophages infected with WNV for 1 h, Q-PCR for TLR3.



Macrophages treated with WNV-E (30 ng for 0, - 3 h,)immunoblot for TLR3. Representative of n=8.

Kong, et al. 2008. *J. Virol.* 82:7613-7623.

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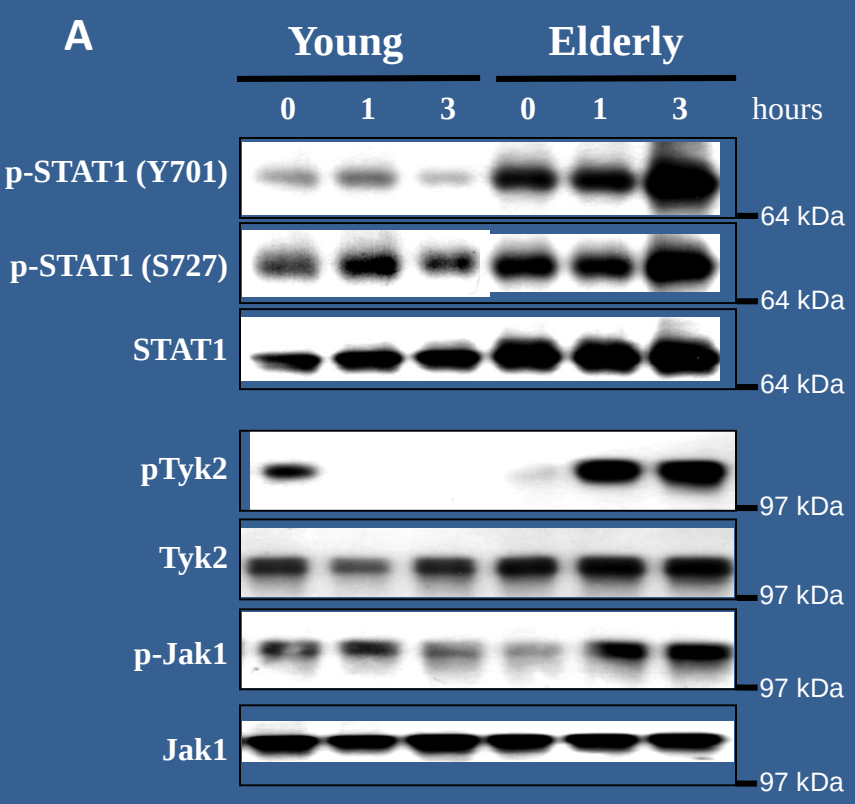
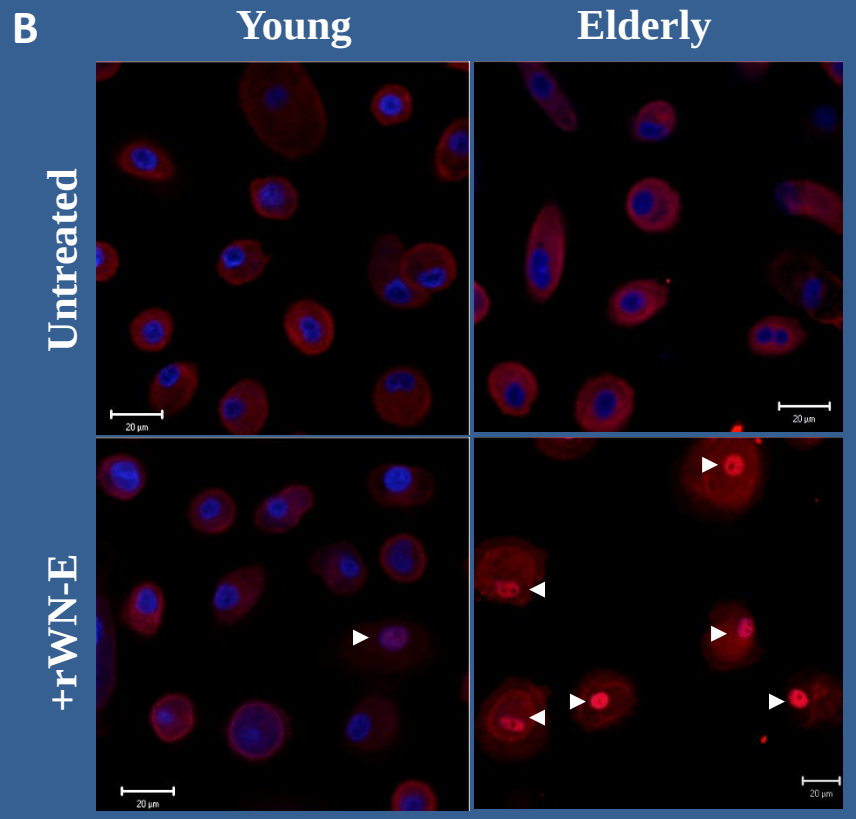
TLR3 dysregulation in the elderly

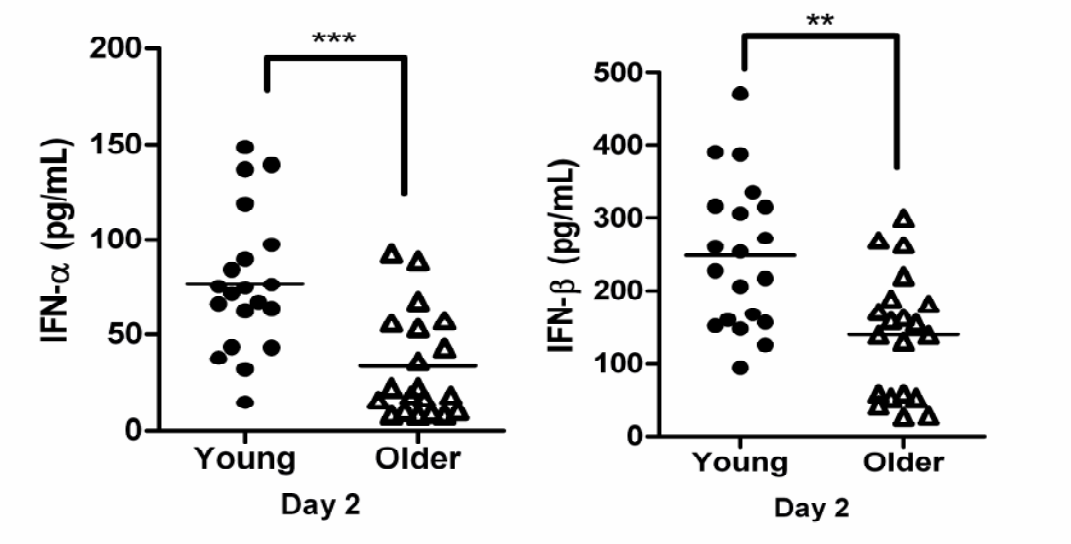




Elderly have ⇑ P-STAT-1, ⇑ nuclear STAT-1, ⇑ viral burden, ⇑ cytokines over 3 days (IL-8, IL-6, TNF, IFNβ; mRNA and ELISA)

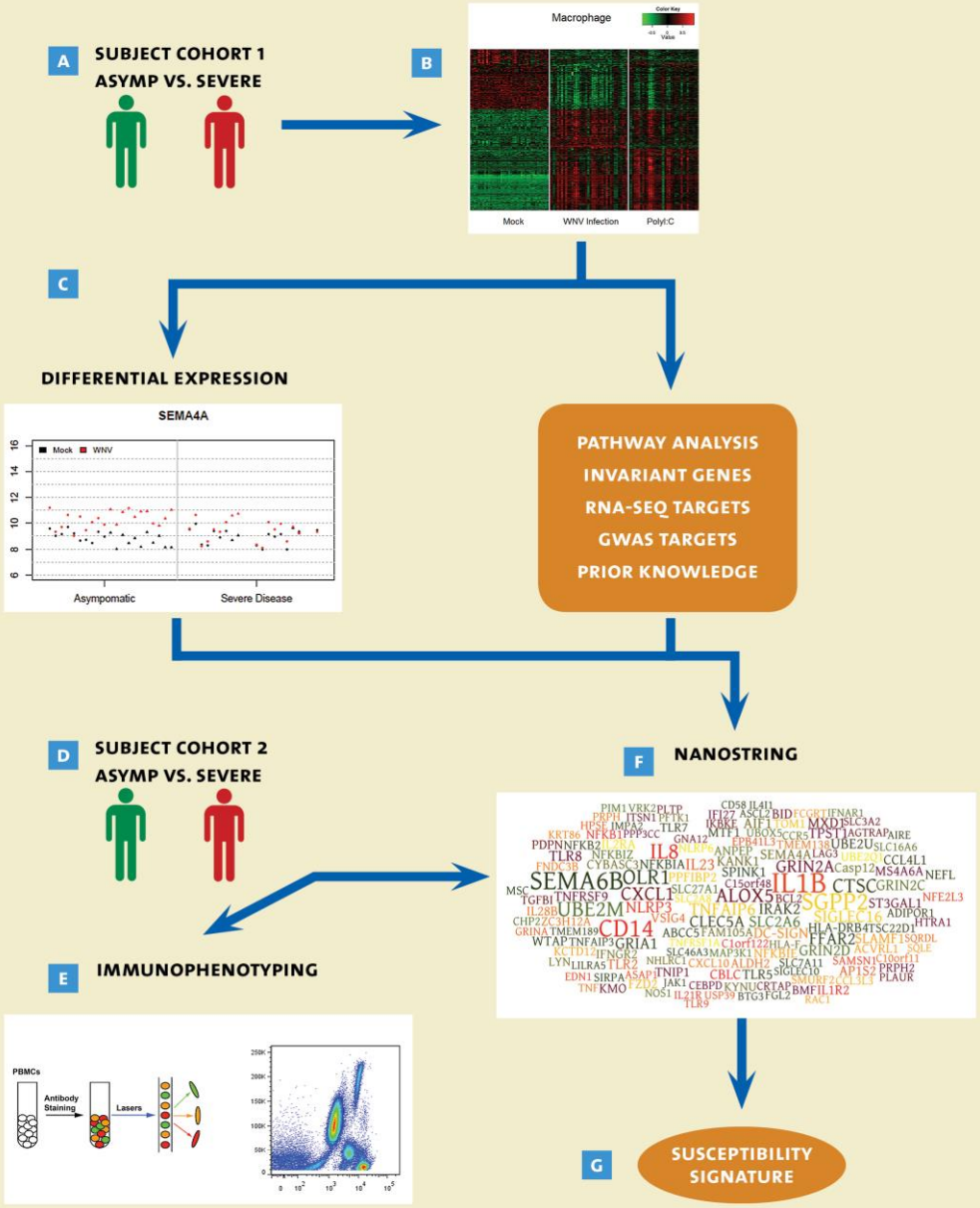
Kong, et al. 2008. Dysregulation of TLR3 impairs the innate immune response to West Nile virus in the elderly. J. Virol. 82:7613-7623.

Impaired interferon signaling in dendritic cells from older donors in response to WNV



Decrease in IFN production in DCs from older donors. DCs were infected with WNV (MOI = 1) for 48 h. IFN-α and IFN-β were quantified by ELISA; * $p < 0.05$, Mann-Whitney n=20/group. Same results from mRNA levels by Q-PCR/actin.

Qian, et al. 2011. J. Infect. Dis,203:1415-1424.



Qian et al. 2015. Systems Immunology reveals markers of susceptibility to West Nile virus infection. *Clin. Vacc. Immunol.* 22:6-16.

Schematic plan of Systems Profiling

Perturbation profiling Approach

- Enroll cohorts with distinct clinical features (old vs young, remission & flare, responders vs non-responders)
- Collect tissue (blood, biopsy)
- Multiple assessments
  - Genomics (GWAS, snps)
  - Phenotyping (FACS, CyTOF)
  - Transcriptional profile (microarray, Nanostring, RNASeq)
  - Functional assays (e.g., antibody titers, cytokines, effector molecules, reactive oxygen species)
- Computational analysis, integrate
  - Differential expression
  - Functional assays
  - Clinical parameters
  - PCA
  - Deconvolution
  - Modeling
- Enroll validation cohort to refine model

Natural Killer (NK) Cells in Stratified WNV Cohort

NK cells may play a role in susceptibility to WNV

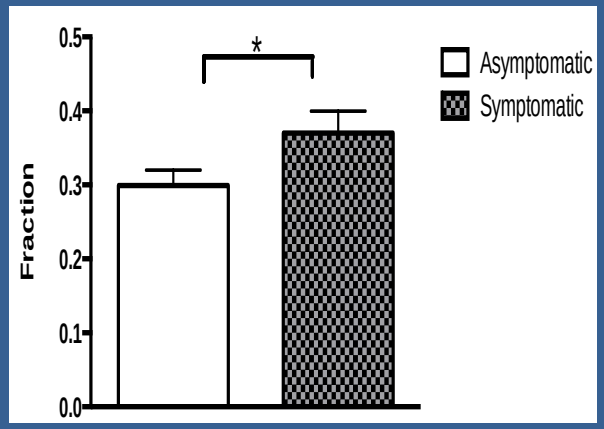
NK cells are innate-like lymphocytes

- defend against viral infections, tumors
- early cell types at inflammatory sites

Activating and inhibitory receptors see:

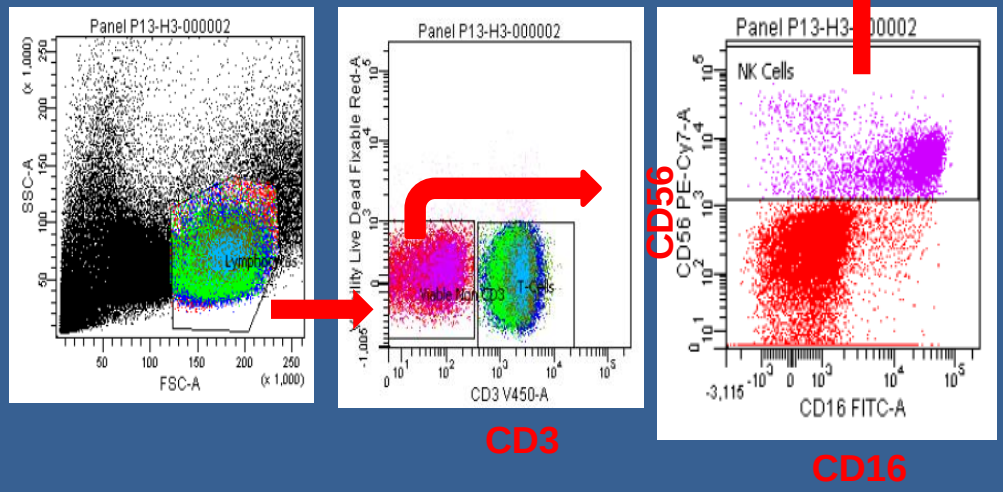
- MHC I molecules
- stressed cells
- pathogen-infected cells
- tumors cells

- Functional **responses**
  - Degranulation: perforin, granzymes
  - Production of cytokines and chemokines: IFNγ, TNFα, MIP-1β
  - Antibody-dependent cellular cytotoxicity (ADCC): CD16 (FcR)-mediated endocytosis of IgG-coated cells



NK cells elevated in severe WNV cohort

PBMCs from WNV subjects were labeled for NK cell markers in multiparameter flow panel. N=21 asymptomatic, n=40 severe. * p<0.05.



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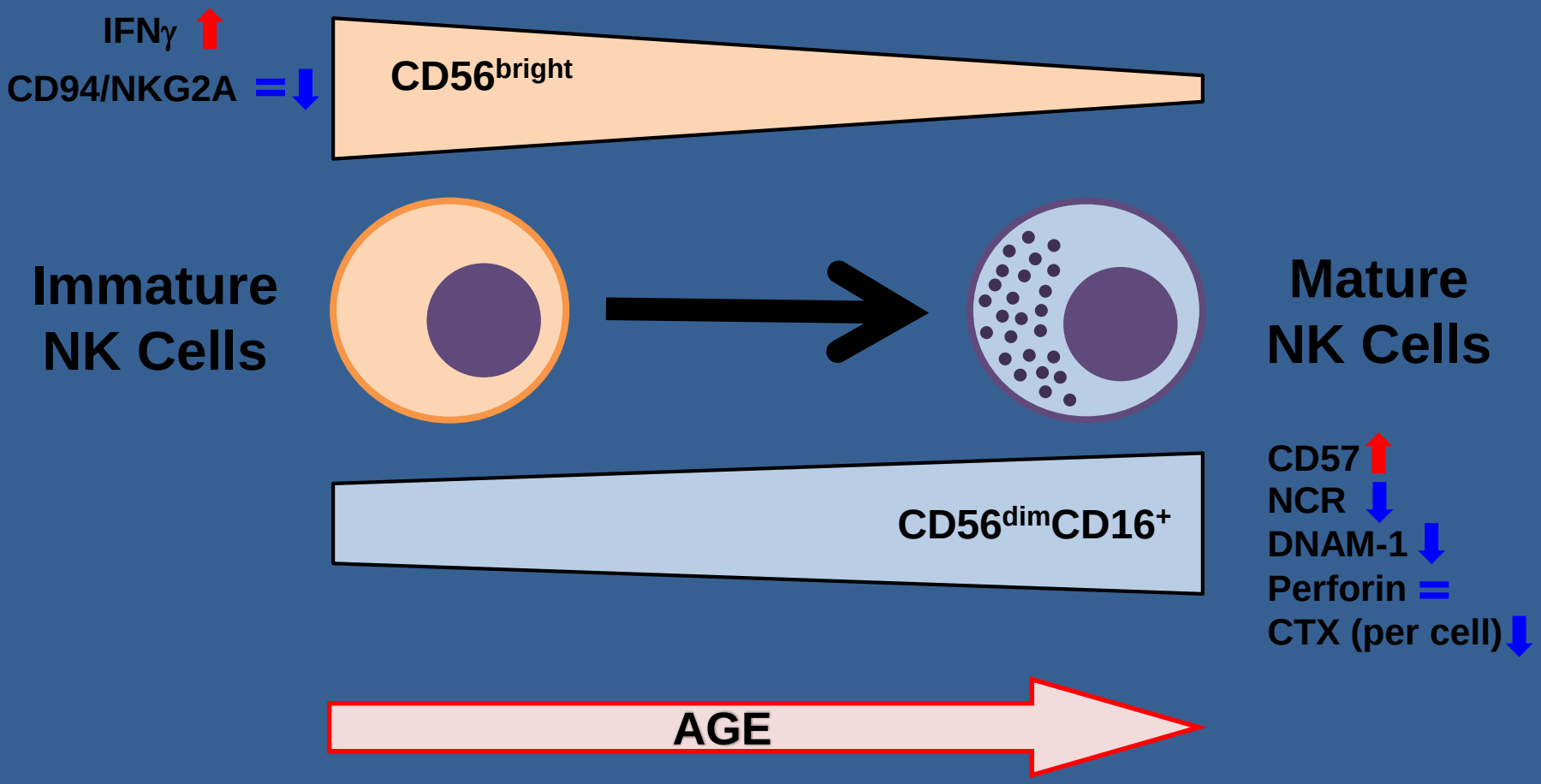
NK Cells in Aging



The phenotypic and functional changes of NK cells in aging are not fully elucidated

Solana R., et al., *Semin Immunol.* 2012
Hazeldine J., & Lord J.M., *Ageing Res Rev.* 2013
Solana R., et al., *Curr Opin Immunol*. 2014

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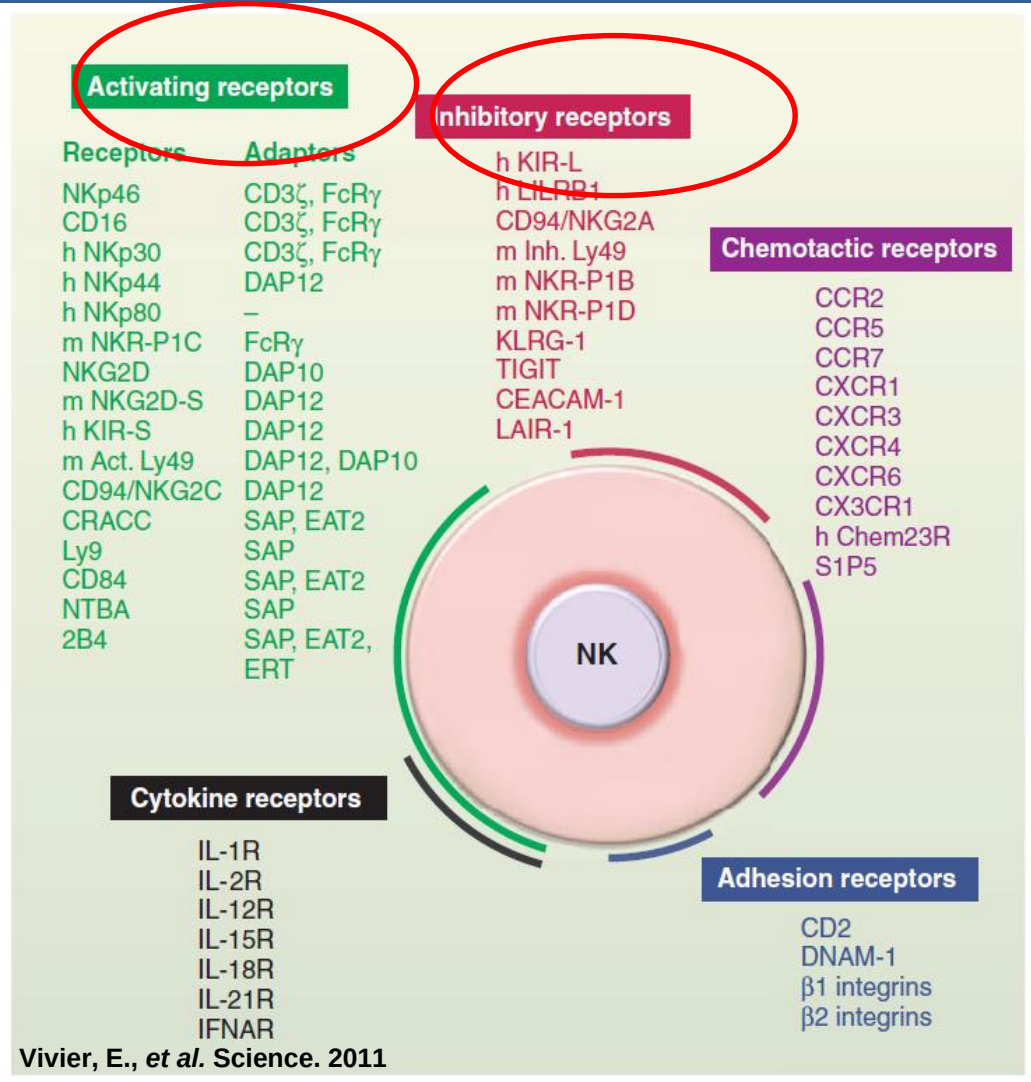
Diversity of NK Cell Receptors



Vivier, E., *et al.* Science. 2011

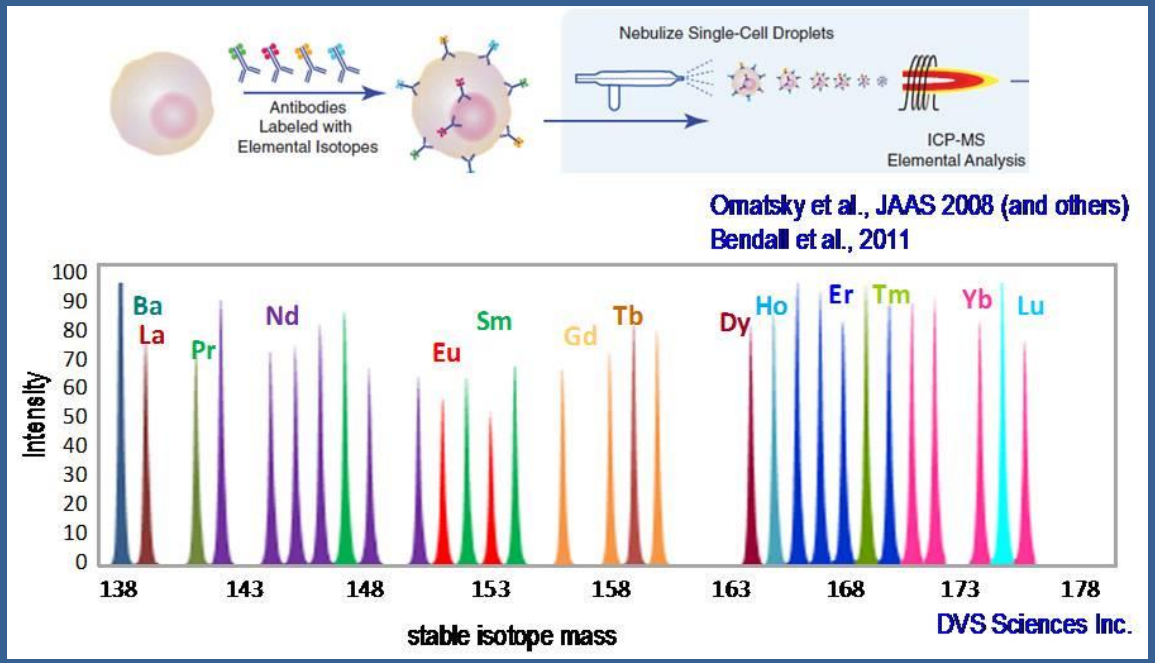
Overall diversity

- *28 NK cell receptors*
- *2^{28} or 268,435,456 combinations*
- *Range 6000-30,000 per individual*
- *median 15,000*

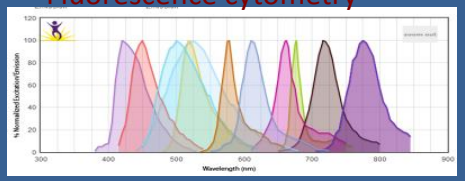
Horowitz, A. et al, Sci Tr Med 2013

NK cell Experimental Procedure

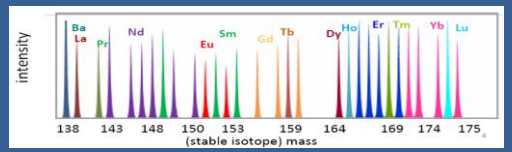
- Healthy younger and older subjects (n= 28)
- CyTOF panel of phenotype (23 markers) and functionality (10 markers)
- NK subsets were assessed at baseline and after infection in vitro with WNV

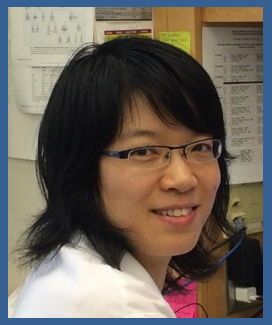


Fluorescence cytometry



Mass cytometry





Yi Yao, PhD

Isotope	Ab
Qdot - Cd	HLA-DR
127I	IdU
141Pr	(CD107a)APC
142Nd	DNAM-1
143Nd	CD4
144Nd	CD8
145Nd	CD57(IgM)
146Nd	PD-1
147Sm	CD3-UCHT1
148Nd	gdTCR
149Sm	CD16
150Nd	MIP-1b
151Eu	GMCSF
152Sm	TNF-a
153Eu	KIR2DS4
154Sm	LILRB1
155Gd	NKp46
156Gd	NKG2D
157Gd	NKG2C
158Gd	CD94
159Tb	CD7
160Gd	CD69
161Dy	NKp30
162Dy	CD33
163Dy	KIR3DL1
164Dy	Anti-WNV(4G2)
165Ho	2B4
166Er	KIR2DL1
167Er	Perforin
168Er	CD19
169Tm	NKp44
170Er	KIR2DL3
171Yb	NKG2A
172Yb	IL-10
173Yb	CD14
174Yb	CD56
175Lu	IL-17A
176Yb	IFN-g
195Pt	Cisplatin

*Metal-conjugated antibodies from **Dr. Catherine Blish** (Stanford)*

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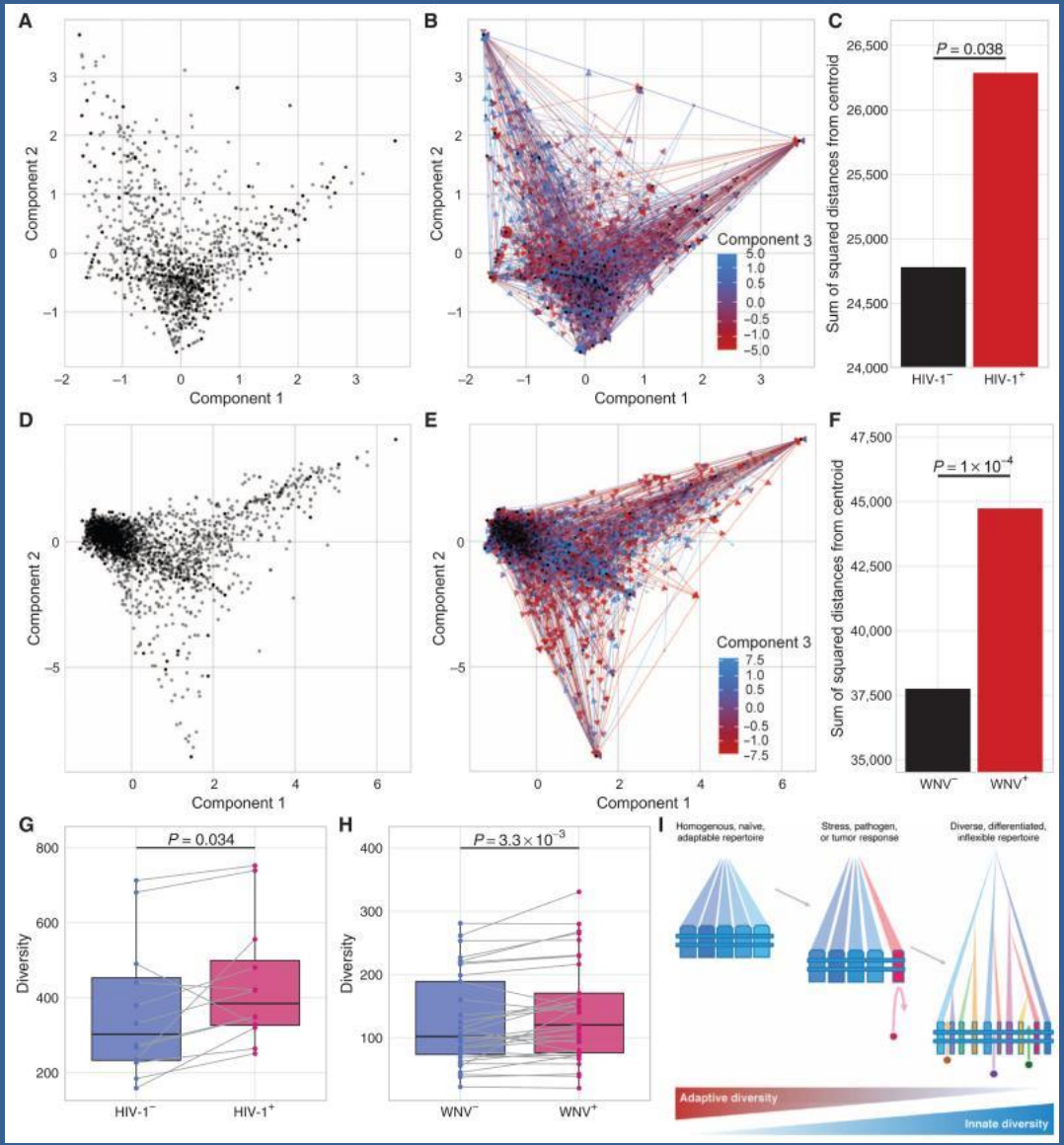
Human NK cell repertoire diversity reflects immune experience and correlates with viral susceptibility



Exposure to virus-infected cells augments NK diversity

Correspondence analysis of Boolean phenotypes for PCA of phenotypes (mock vs infected) for:

- HIV-1 (A to C, *n* = 12), 11,523 total phenotypes
- WNV (D to F, *n* = 33) 8,397 total phenotypes

Sum of squared distances to centroid of point cloud in NK cells (C, F). Permutation test (10,000×) on sum of squared distances from centroid: HIV-1, $P = 0.038$; WNV, $P = 1.0 \times 10^{-4}$.

Model for NK cell diversity:

A naïve NK repertoire is an effective fence for infection prevention. As the NK repertoire encounters novel pathogens over the course of a lifetime, it diversifies with each response mounted. Its increasingly branched, diffuse nature increases the chance that a newly encountered virus will penetrate the barrier.

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Straus-Albee et al, Sci.Transl. Med. (2015) 7:297ra115

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NIAID/DAIT

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