

Glycosylation des anticorps

du fondamental à l'appliqué

**Dr Thierry VINCENT
Laboratoire d'Immunologie
Hôpital St ELOI - CHU de Montpellier**

Are we sure to look for the good antibodies ??

**Are we able to distinguish
pathogenic vs non pathogenic antibodies ??**

immunosuppressive / anti-inflammatory Abs

Tolerance induction with T cell-dependent protein antigens induces regulatory sialylated IgGs

Carolin M. Oefner, MSc,^{**} André Winkler, MSc,^{**} Constanze Hess, PhD,^{**} Alexandra K. Lorenz, BSc,^a Vivien Holecska, Tech,^a Melanie Huxdorf, MSc,^a Tim Schommartz, MSc,^b Dominique Petzold, MSc,^{b,c} Josephine Bitterling, Tech,^b Anna-Lena Schoen, MSc,^b Alexander D. Stoehr, PhD,^a Dana Vu Van, MSc,^{d,e} Yasemin Darcan-Nikolaisen, PhD,^f Véronique Blanchard, PhD,^g Inken Schmutte, MSc,^g Yves Laumonier, PhD,^g Heike A. Ströver, MSc,^g Ahmed N. Hegazy, MD,^{h,i} Susanne Eiglmeier, PhD,^a Carolin T. Schoen, PhD,^a Maria M. M. Mertes, PhD,^a Christoph Loddenkemper, MD,^{j,k} Max Löhning, PhD,^h Peter König, MD,^{l,m} Arnd Petersen, PhD,ⁿ Elke O. Luger, PhD,^o Mattias Collin, PhD,^p Jörg Köhl, MD,^{q,m} Andreas Hutloff, PhD,^{d,e} Eckard Hamelmann, MD,^{f,q} Markus Berger, PhD,^o Hedda Wardemann, PhD,^r and Marc Ehlers, PhD^{a,b,m} *Berlin, Lübeck,*

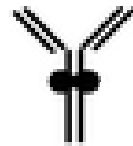
J Allergy Clin Immunol.2012 Jun;129(6):1647-55

T cell-independent B cell activation induces immunosuppressive sialylated IgG antibodies

Constanze Hess,¹ André Winkler,^{1,2} Alexandra K. Lorenz,¹ Vivien Holecska,¹ Véronique Blanchard,³ Susanne Eiglmeier,¹ Anna-Lena Schoen,² Josephine Bitterling,² Alexander D. Stoehr,¹ Dominique Petzold,^{2,3} Tim Schommartz,² Maria M.M. Mertes,¹ Carolin T. Schoen,¹ Ben Tiburzy,⁴ Anne Herrmann,⁴ Jörg Köhl,^{4,5} Rudolf A. Manz,⁴ Michael P. Madaio,⁶ Markus Berger,³ Hedda Wardemann,⁷ and Marc Ehlers^{1,2}

***J Clin Invest.* 2013;123(9):3788–3796.**

Effector functions of IgG are regulated by their Fc N-linked glycosylation

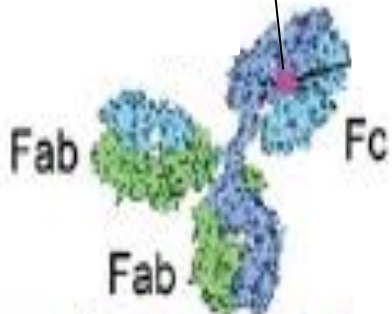


N-glycosylation

Effector function

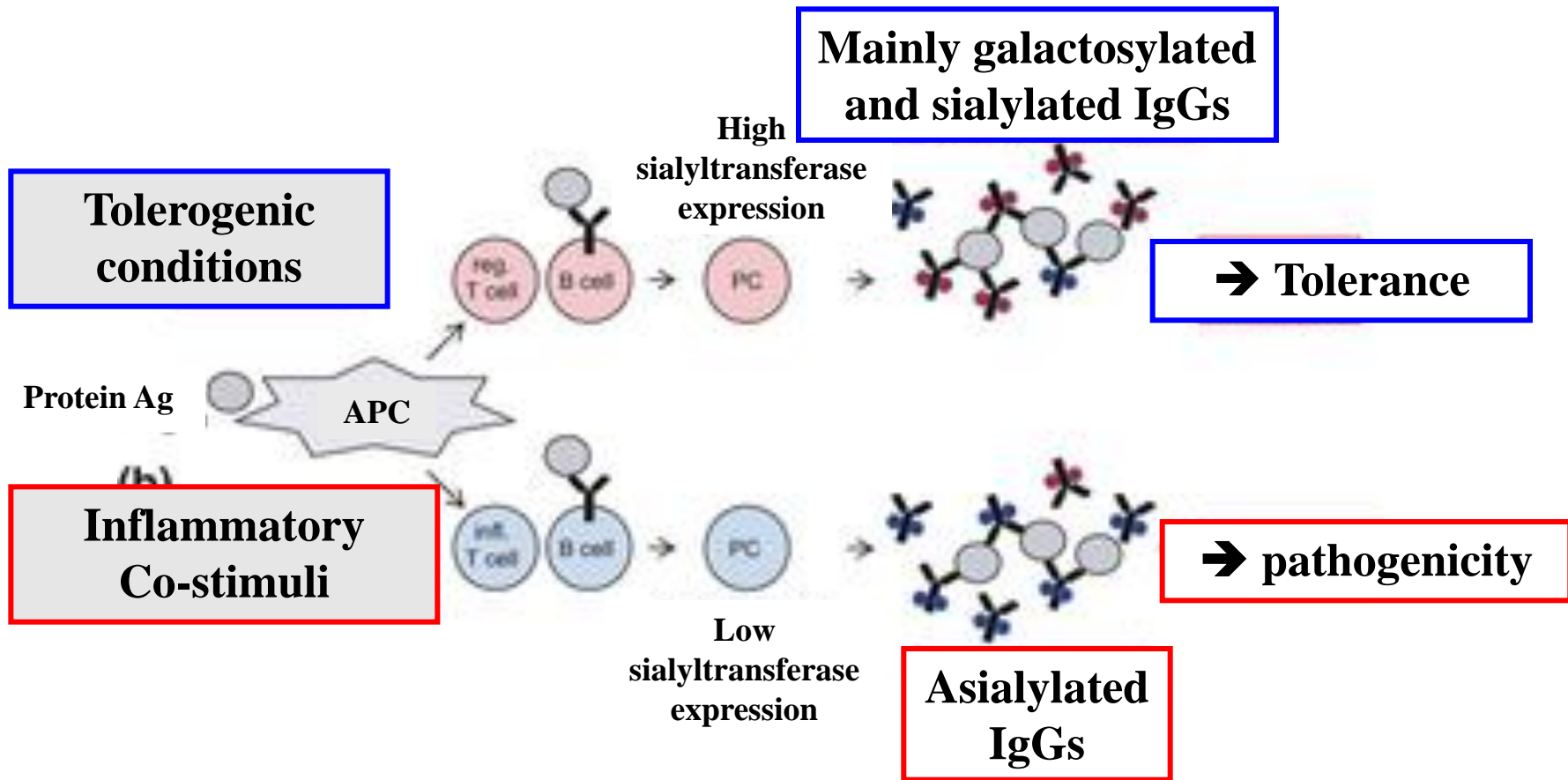
Receptors

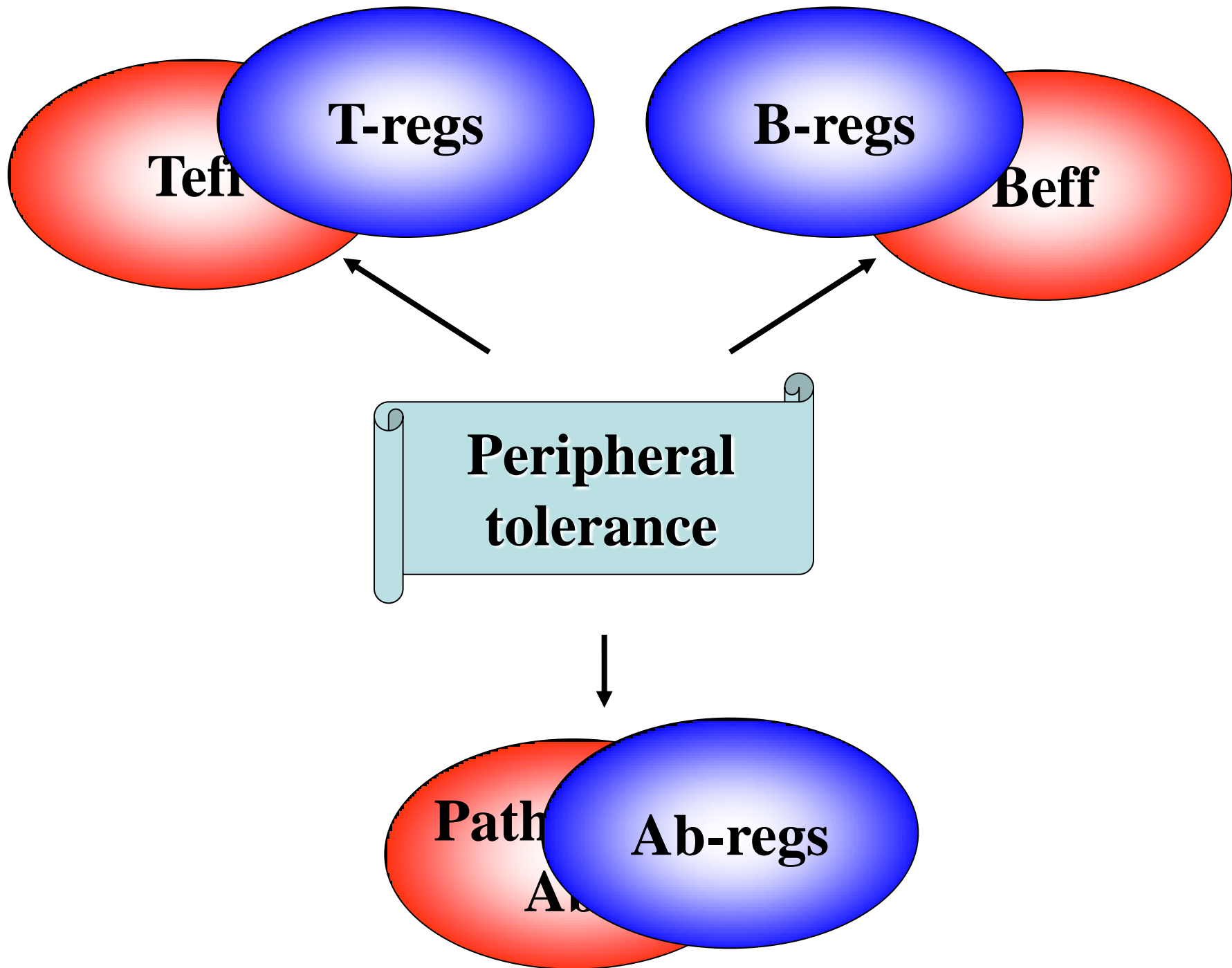
Glycosylation site
(Asn²⁹⁷)



Asn ²⁹⁷		(G1S1, G2S1), G2S2	Immuno- suppressive	DC-SIGN
Asn ²⁹⁷		(G1), G2		FcγRIIb and dectin-1
Asn ²⁹⁷		G0	Pathogenic	Activating FcγRs and Mannose receptor

■, □: GlcNAc ▲: Fucose ●: Man ●: Galactose ◆: Sialic acid

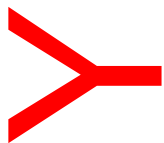
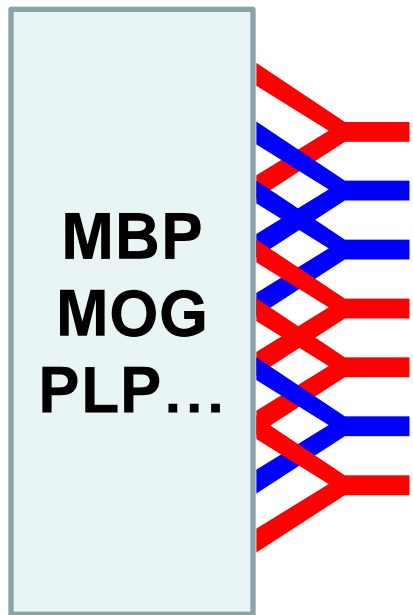




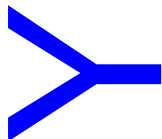
Applications

diagnostic / pronostic

Current assays

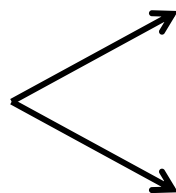
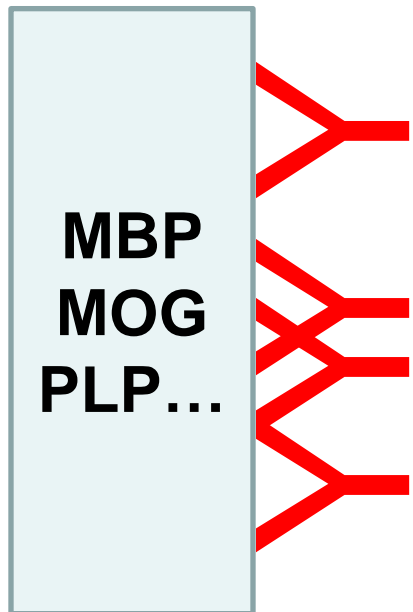


asialylated Ab



sialylated Ab

Next generation



Patients

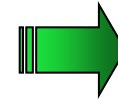
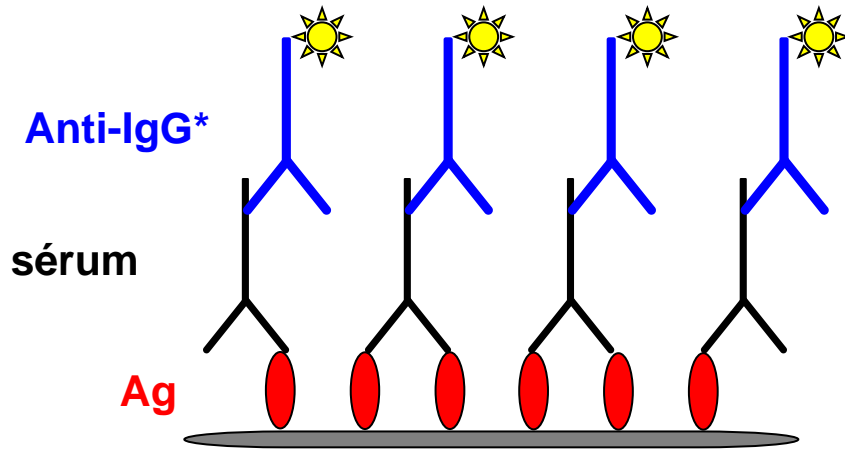
relapse

controls

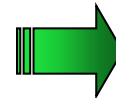
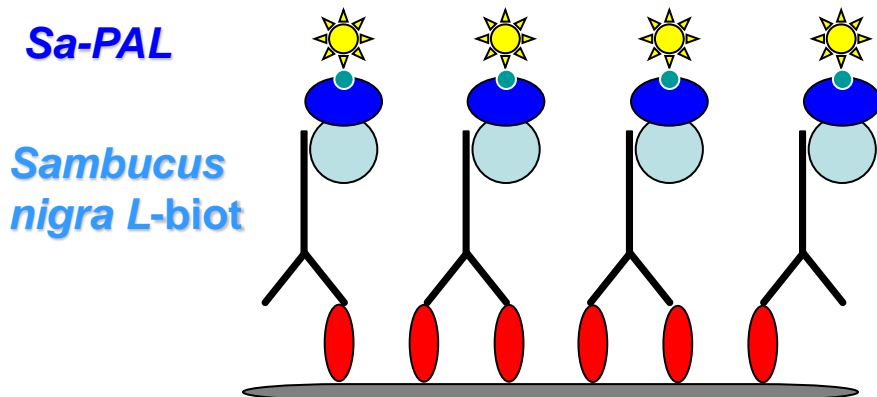
remission

Sambucus nigra Lectin

→ se lie préférentiellement à l'acide sialique attachée à un galactose terminal



Taux d'auto-Ac

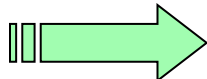
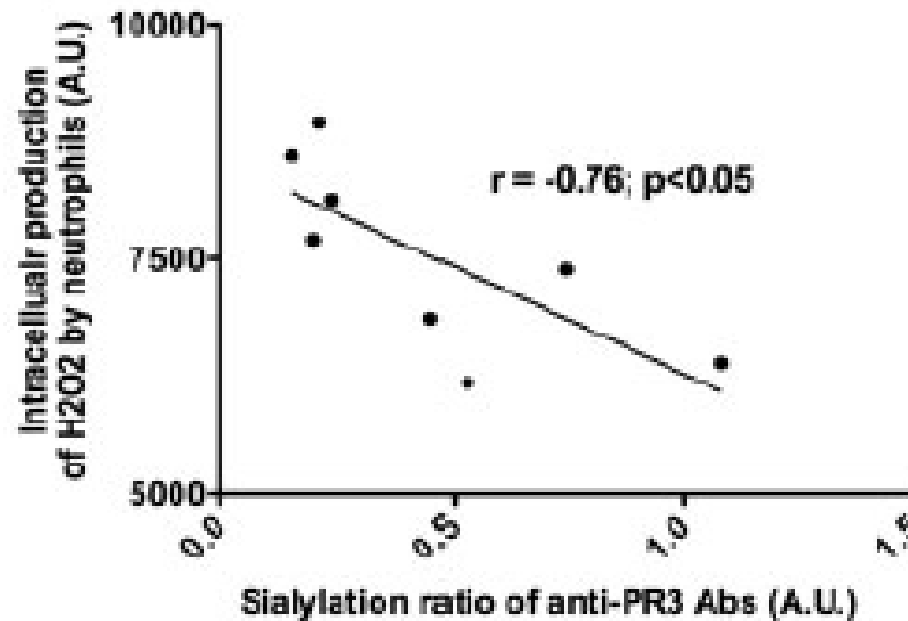


Niveau de sialylation

Maladie de Wegener et sialylation des Ac anti-PR3

Mesure du ratio de sialylation = acide sialique / anti-PR3

PNN + IgG purifiés
→ Mesure de la production intracellulaire d'H₂O₂

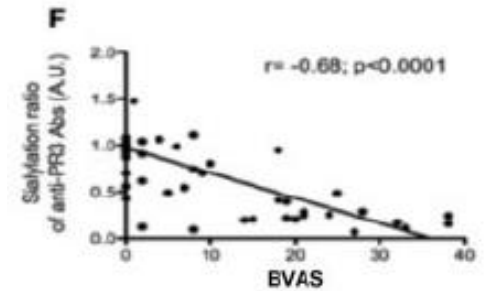
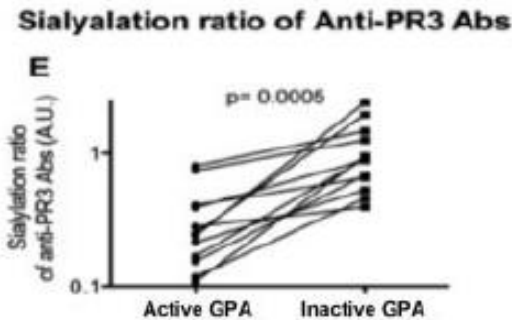
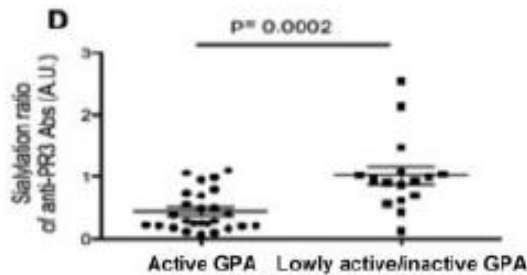
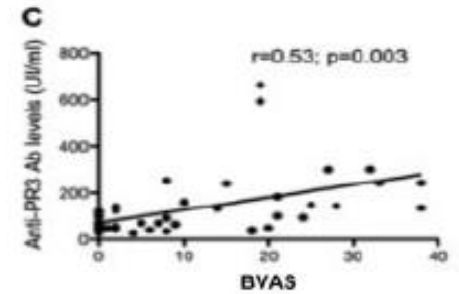
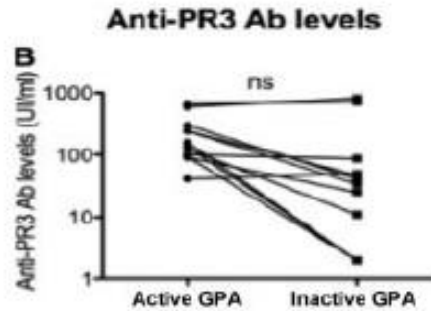
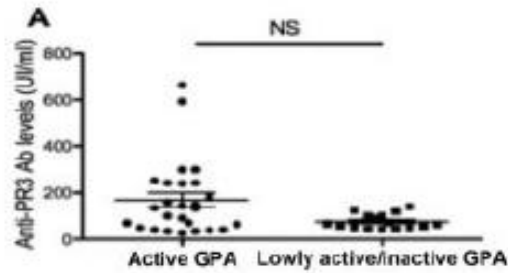


Ac pathogènes = hyposialylés

Maladie de Wegener et sialylation des Ac anti-PR3

Ac anti-PR3

Sialylation ratio



Corrélation avec l'activité de la maladie:

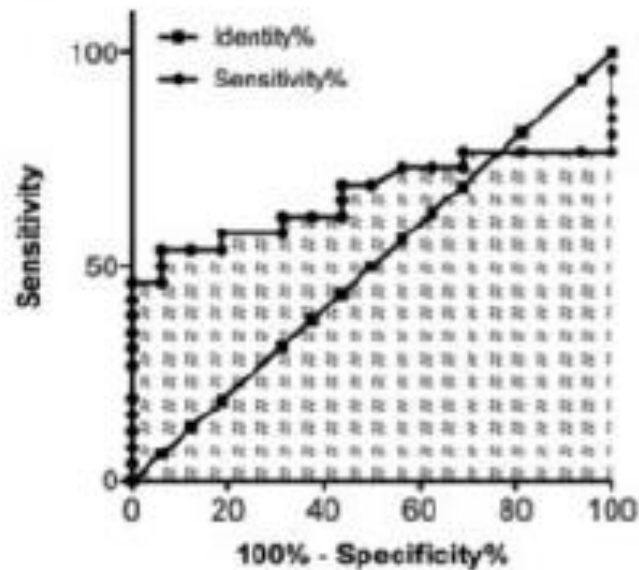
Sialylation ratio >> anti-PR3

Maladie de Wegener et sialylation des Ac anti-PR3

Anti-PR3

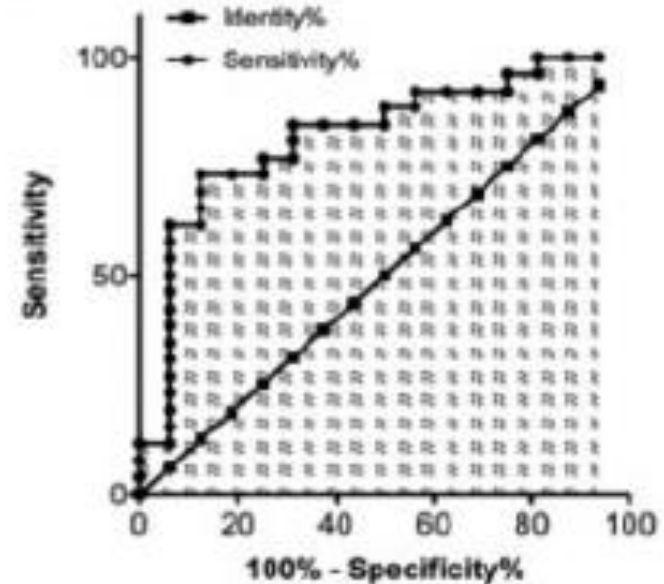
Sialylation ratio

A



	Results
Area under the ROC curve	
Area	0.6647
Std. Error	0.06471
95% confidence interval	0.4986 to 0.8307
P value	0.07609

B



	Results
Area under the ROC curve	
Area	0.8197
Std. Error	0.06951
95% confidence interval	0.6834 to 0.9560
P value	0.0005762

ORIGINAL ARTICLE

Rituximab versus Cyclophosphamide for ANCA-Associated Vasculitis

John H. Stone, M.D., M.P.H., Peter A. Merkel, M.D., M.P.H., Robert Spiera, M.D.,
Philip Seo, M.D., M.H.S., Carol A. Langford, M.D., M.H.S.,
Gary S. Hoffman, M.D., Cees G.M. Kallenberg, M.D., Ph.D.,
E. William St. Clair, M.D., Anthony Turkiewicz, M.D., Nadia K. Tchao, M.D.,
Lisa Webber, R.N., Linna Ding, M.D., Ph.D., Lourdes P. Sejismundo, R.N., B.S.N.,
Kathleen Mieras, C.C.R.P., David Weitzenkamp, Ph.D., David Ikle, Ph.D.,
Vicki Seyfert-Margolis, Ph.D., Mark Mueller, B.S., C.C.R.P., Paul Brunetta, M.D.,
Nancy B. Allen, M.D., Fernando C. Fervenza, M.D., Ph.D., Duvuru Geetha, M.D.,
Karina A. Keogh, M.D., Eugene Y. Kissin, M.D., Paul A. Monach, M.D., Ph.D.,
Tobias Peikert, M.D., Coen Stegeman, M.D., Ph.D., Steven R. Ytterberg, M.D.,
and Ulrich Specks, M.D., for the RAVE-ITN Research Group*

NEJM July 2010; 363(3): 221

Cohorte RAVE (Mayo clinic)

- 131 patients GPA (Wegener) avec ANCA+
- Traités par Rituximab ou cyclophosphamide
- suivi longitudinal : Jo, M1, M4, M6, M9, M12, M15, M18 and flares (1179 sérums)

Quantification des anti-PR3 sérique (ELISA Euroimmun)

Mesure du niveau de sialylation

→ Calcul du ratio anti-PR3 / acide sialique (SR-PR3)

→ Comparaison avec données cliniques: PR3 versus ratio

➡ SR-PR3 meilleurs biomarqueur que anti-PR3...???

Applications thérapeutiques

Application aux Ig polyvalentes Intraveineuses (IGIV)

→ l'effet thérapeutique principal des IGIV passe par leurs portions Fc

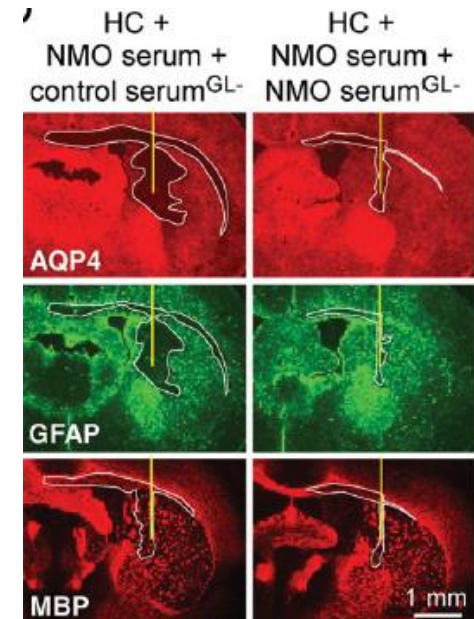
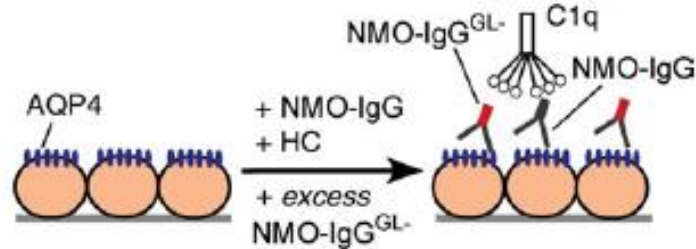
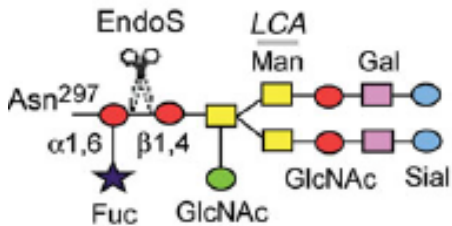
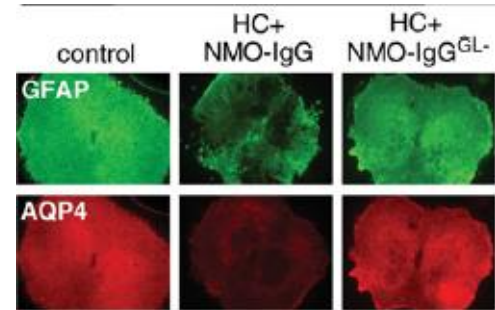
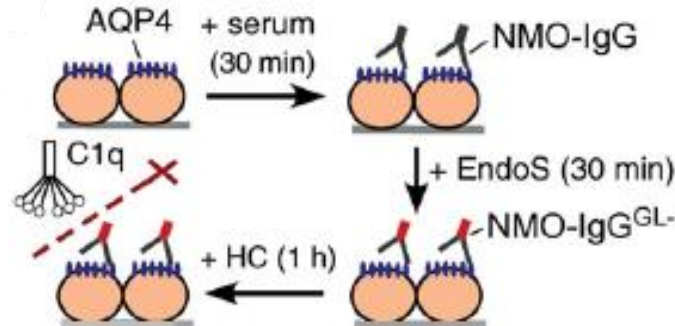
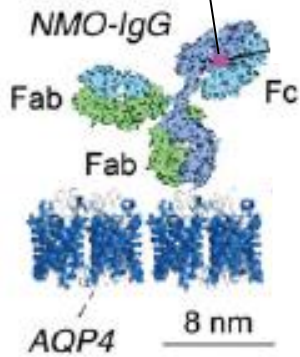
→ le principal composant anti-inflammatoire des IgIV est la fraction sialylée des IgG qui ne représente que 10% des IgIV

→ d'où la nécessité de fortes doses pour obtenir un effet immunomodulateur.

→ Préparations enrichies en IgG sialylées ou fragments Fc recombinants sialylés

Deglycosylated NMO-IgG

Glycosylation site
(Asn²⁹⁷)



Endoglycosidase S (EndoS)

Secrété par *Streptococcus pyogenes*

Clive sélectivement les sucres liés à Asn de la chaîne lourde des IgG

Pas d'action sur les autres classes d'Ig ou d'autres glycoprotéines

→ **converti les auto-Ac pathogènes en Ac thérapeutiques bloquants**

→ **aphérèse thérapeutique avec surface coatée avec EndoS**

→ **injection systémique ?**

→ **Vascularites à ANCA, PTI, AHAI, LES, NMO...**

CONCLUSION

→ Discriminer les Ac pathogènes / non pathogènes

- Diagnostic: ↗ spécificité

- Pronostic: biomarqueurs

→ Modifier les fonctions effectrices des Ac

- MAI: ↘

- Cancer: ↗