Induction of antigen-specific tolerance with peptide epitopes

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Antigen-directed therapy of hypersensitivity diseases

- In 1911, Drs John Freeman and Leonard Noon published an account of a novel treatment for hay fever. Their method of desensitisation consisted of injecting increasing doses of an extract of pollen subcutaneously until the hypersensitivity reaction was diminished or abolished
- “there seems little doubt that there has been a distinct amelioration of symptoms. This improvement took several forms; a greater freedom from attack, the attack not so bad as in former years, and the attack sooner over, the constitutional disturbance not so great”

1. Noon, L. Prophylactic innoculation against hay fever. Lancet i, 1572-1573. 1911

2. Larche, M. and Wraith, D.C. Peptide Vaccines for Allergic and Autoimmune Diseases Nature Medicine (2005) 11: 69-76

Application of antigen-specific immunotherapy

- **ALLERGY**
- **AUTOIMMUNITY**
- **Aberrant immune responses to therapeutic proteins e.g. factor VIII intolerance in hemophilia A**

Nature of the antigen

- Intact antigen can activate mast cells and basophils by cross-linking IgE
- Intact antigen can stimulate antibody secretion (MOG in EAE)
- Intact antigen given orally can stimulate cytotoxic T lymphocytes (Insulin in diabetes)
- Use CD4 T cell epitopes*

*Harrison, L. C. and D. Hafler (2000). "Antigen-specific therapy for autoimmune disease." Current Opinion in Immunology **12**: 704-711.

Response to self-antigens: 1993

Inhibition of experimental autoimmune encephalomyelitis by inhalation but not oral administration of the encephalitogenic peptide: influence of MHC binding affinity

Barbara Metzler and **David C. Wraith**

Cambridge University Department of Pathology, Immunology Division, Tennis Court Road, Cambridge CB2 1QP, UK

International Immunology, Vol. 5, No. 9, pp. 1159–1165

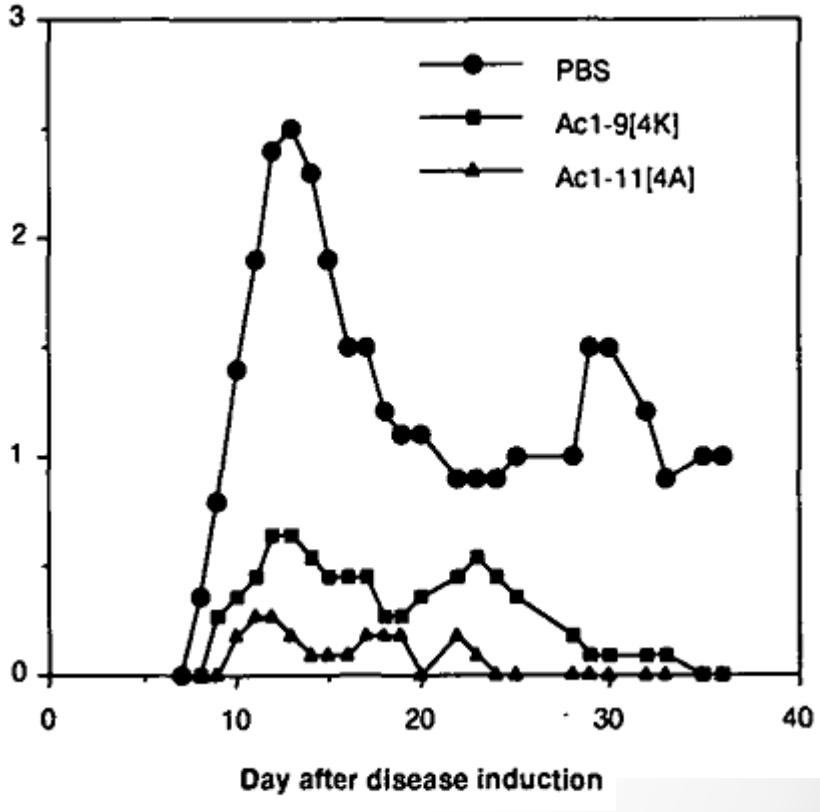


Fig. 4. Effect of peptide inhalation on EAE induced with whole SCH in (PL/J × B10. PL)F_1. Mice were given a single intranasal dose of 100 μg peptide in PBS or PBS alone. At 7 days later all animals were primed with 1mg SCH (otherwise see legend to Fig. 1). (a) $^{a}P < 0.001$, $^{b}P < 0.02$. (b) $^{a}P < 0.05$ for 1 versus 3 and $P < 0.05$ for 2 versus 3, $^{b}P < 0.05$ for 1 versus 2 and $P < 0.02$ for 1 versus 3.

Response to allergens: 1993

Brief Definitive Report

Inhibition of T Cell and Antibody Responses to House Dust Mite Allergen by Inhalation of the Dominant T Cell Epitope in Naive and Sensitized Mice

By Gerard F. Hoyne,* Robyn E. O'Hehir, David C. Wraith,‡ Wayne R. Thomas,* and Jonathan R. Lamb

Journal of Experimental Medicine 178, 1783.

Evidence of 'linked suppression'

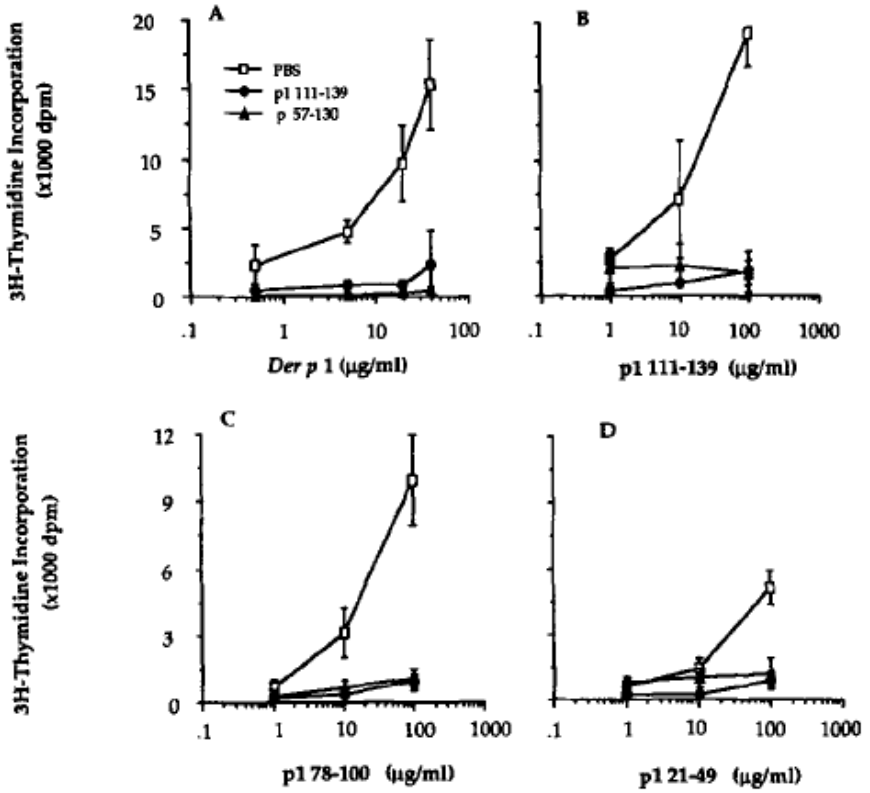


Figure 1. Peptides given intranasally to mice can inhibit T cell responses. Mice were treated with either PBS (□), 100 μg of GEX p57–130 (▲), or p1 111–139 (●) intranasally on three consecutive days and 1 wk later all mice were immunized with 100 μg of Der p 1/CFA. LN cells were collected 7 d later and cultured in vitro with (*A*) Der p 1 protein, (*B*) p1 111–139, (*C*) p1 78–100, or (*D*) p1 21–49 for 24 h. Data shows the mean IL-2 response of five mice per group ± SD.

Autoimmune disease models

Disease	Species	Peptide	Dose/animal & route	Reference
MS	Mouse	MBP Ac1-9	100 μg i.n.	Metzler et al 1993 Burkhart et al 1999
MS	Mouse	MBP Ac1-9	100 μg i.p.	Liu et al 1995
MS	Mouse	PLP 139-151	100 μg i.n.	Anderton et al 1998
MS	Rat	MBP 87-99	5 x 120 μg i.n	Liu, et al 1998
Arthritis	Mouse	Collagen II 245-270	3 x 100 μg i.n.	Chu et al 1999
Arthritis	Rat	HSP60 176-190	3 x 100 μg i.n. or s.c.	Prakken et al 1997
Diabetes	Mouse	4 GAD peptides	200 μg i.n.	Tian et al 1996
Diabetes	Mouse	Insulin 9-23	100 μg i.n. or s.c.	Daniel et al 1996
Diabetes	Mouse	HSP60 p277	50 μg i.p.	Elias et al 1991
AIHA	Mouse	Band 3 861-874	100 μg i.n.	Shen et al. 2003
SLE	Mouse	SmD1 83-119	600 μg i.v./month	Riemekasten et al 2004
Myasthenia	Mouse	3 AChR peptides	50 μg i.n.	Karachunski et al 1997
Neuritis	Rat	P0 180-199	10 x 6 μg i.n.	Zou et al 1999

Apitopes: tolerogenic T-cell epitopes

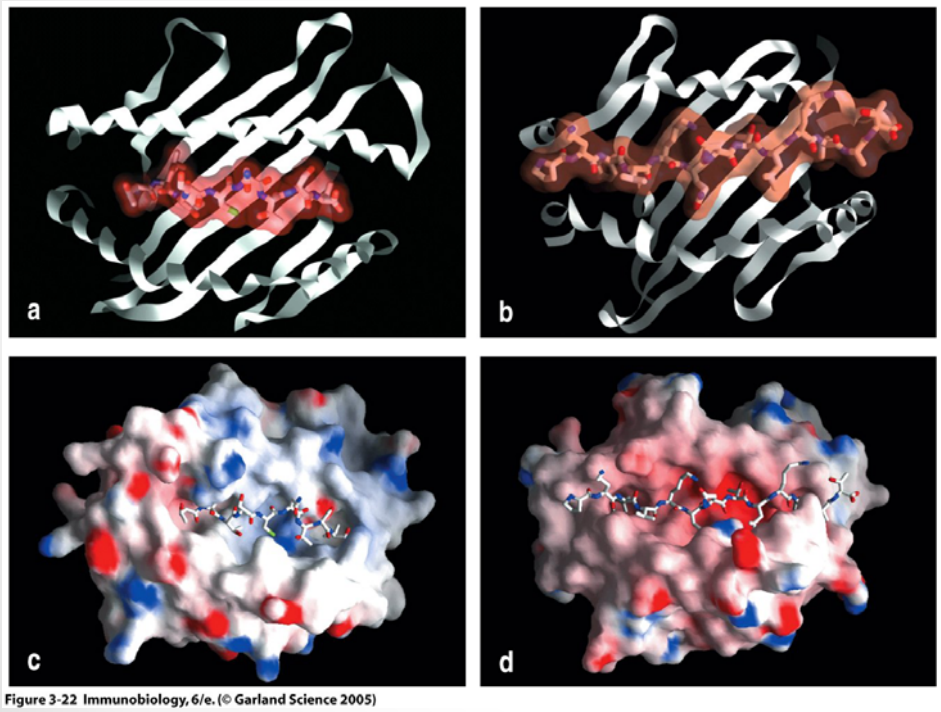


Figure 3-22 Immunobiology, 6/e. (© Garland Science 2005)

Influence of a dominant cryptic epitope on autoimmune T cell tolerance

Stephen M. Anderton[1], Nicholas J. Viner[2], Philip Matharu[2], Pauline A. Lowrey[2] and David C. Wraith[2]

Nature Immunology 3, 175.2002

- Not all T cell epitopes induce tolerance
- Peptides must be designed to mimic naturally processed epitopes
- Such peptides are defined as antigen processing independent epitopes or apitopes

Mode of action

- Apitopes bind directly to MHC at the surface of antigen presenting cells
- When presented in the absence of ‘danger’ signals, apitopes induce immunological tolerance
- Dendritic cells are more important than B cells as tolerogenic antigen presenting cells
- What is the nature of the induced immunological tolerance?

Treg cells: Tg4 transgenic TCR vs Ac1-9 of MBP

Natural Regulators

Spleen CD4 cells
FACS sorted:
92-95% CD25-ve
5-8% CD25+ve

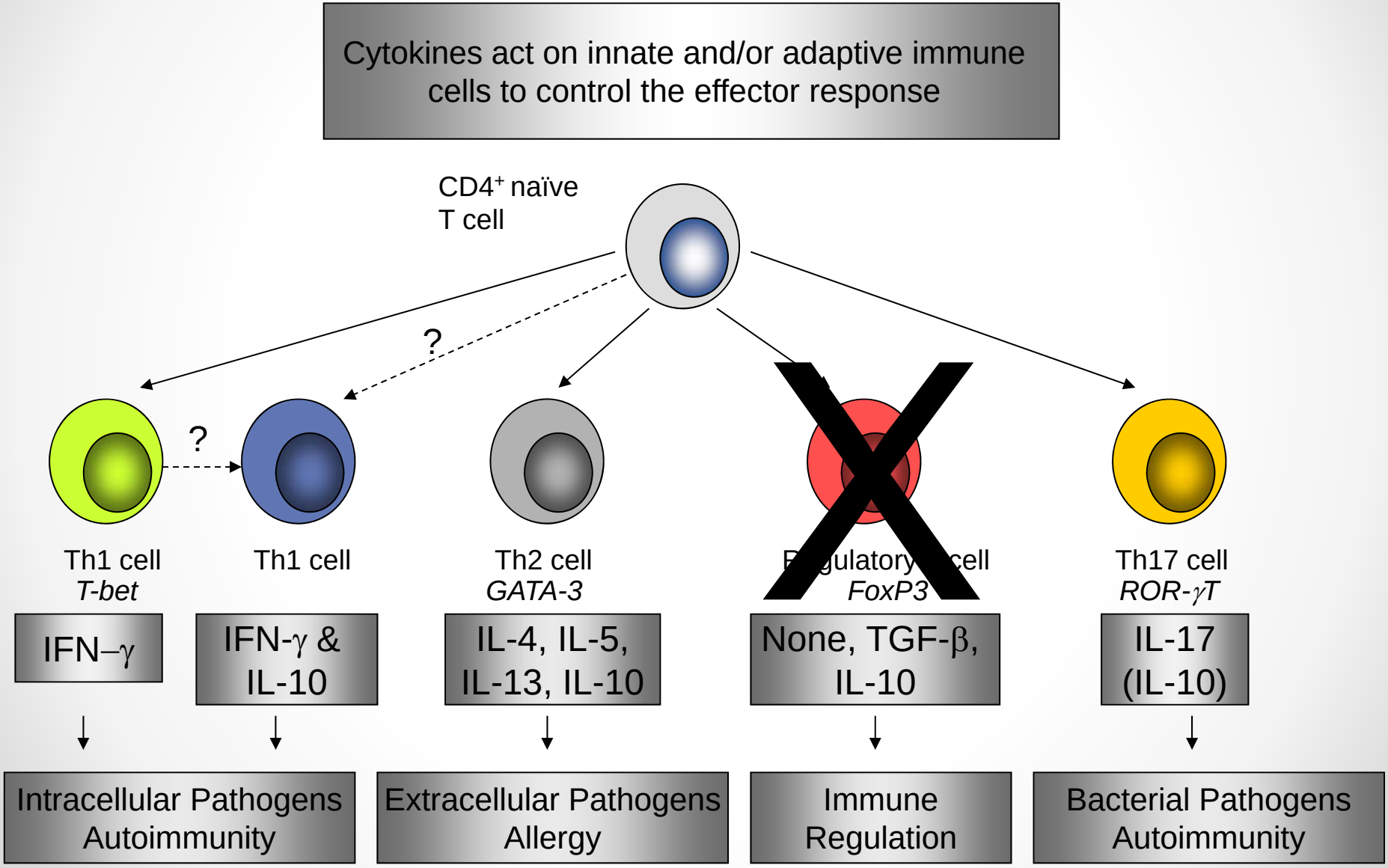
Induced Regulators

Intranasal
Peptide 2x
Per week

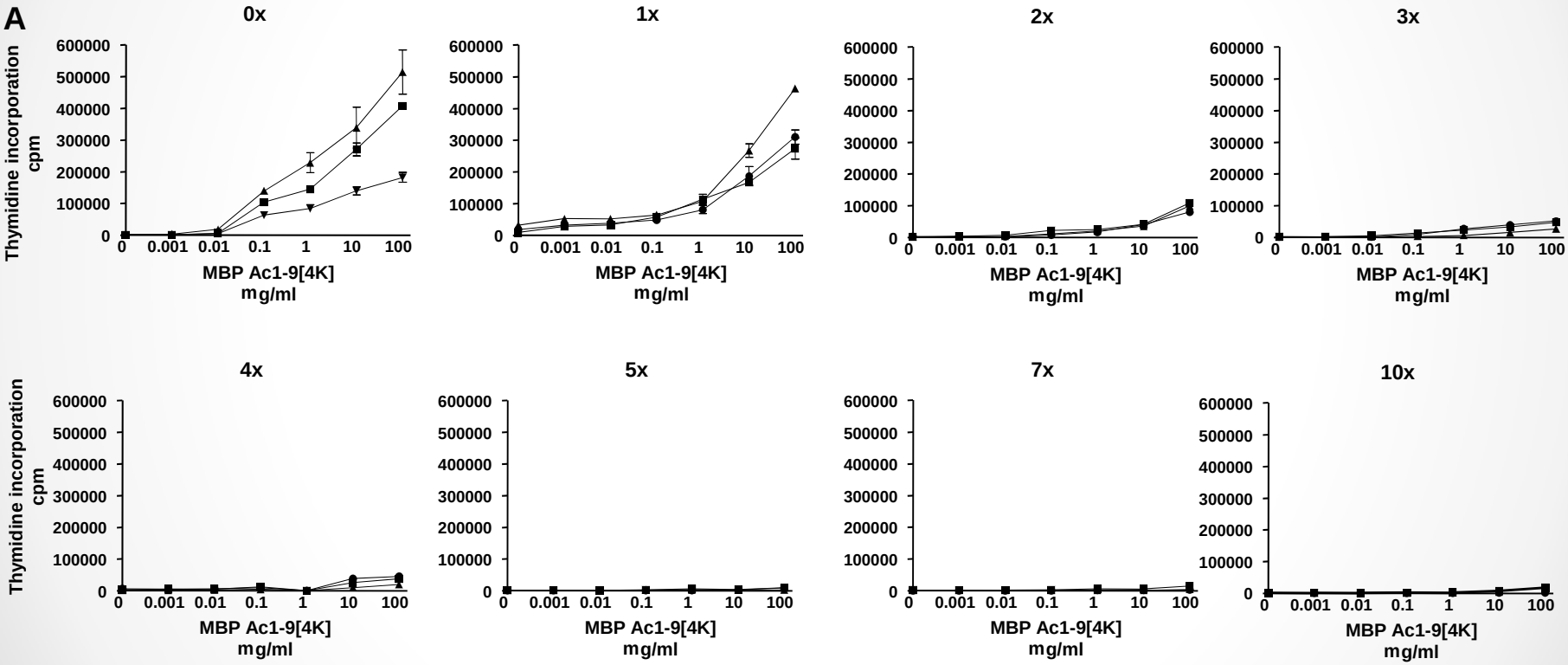
- injection of myelin in CFA induces EAE
- 5-10 doses of intranasal peptide prevents induction of EAE
- protection abrogated by anti-IL-10

Burkhart et al. Int. Immunol. (1999) 11, 1625
Sunstedt et al. J. Immunol. (2003) 170, 1240

IL-10 secreting T-cells



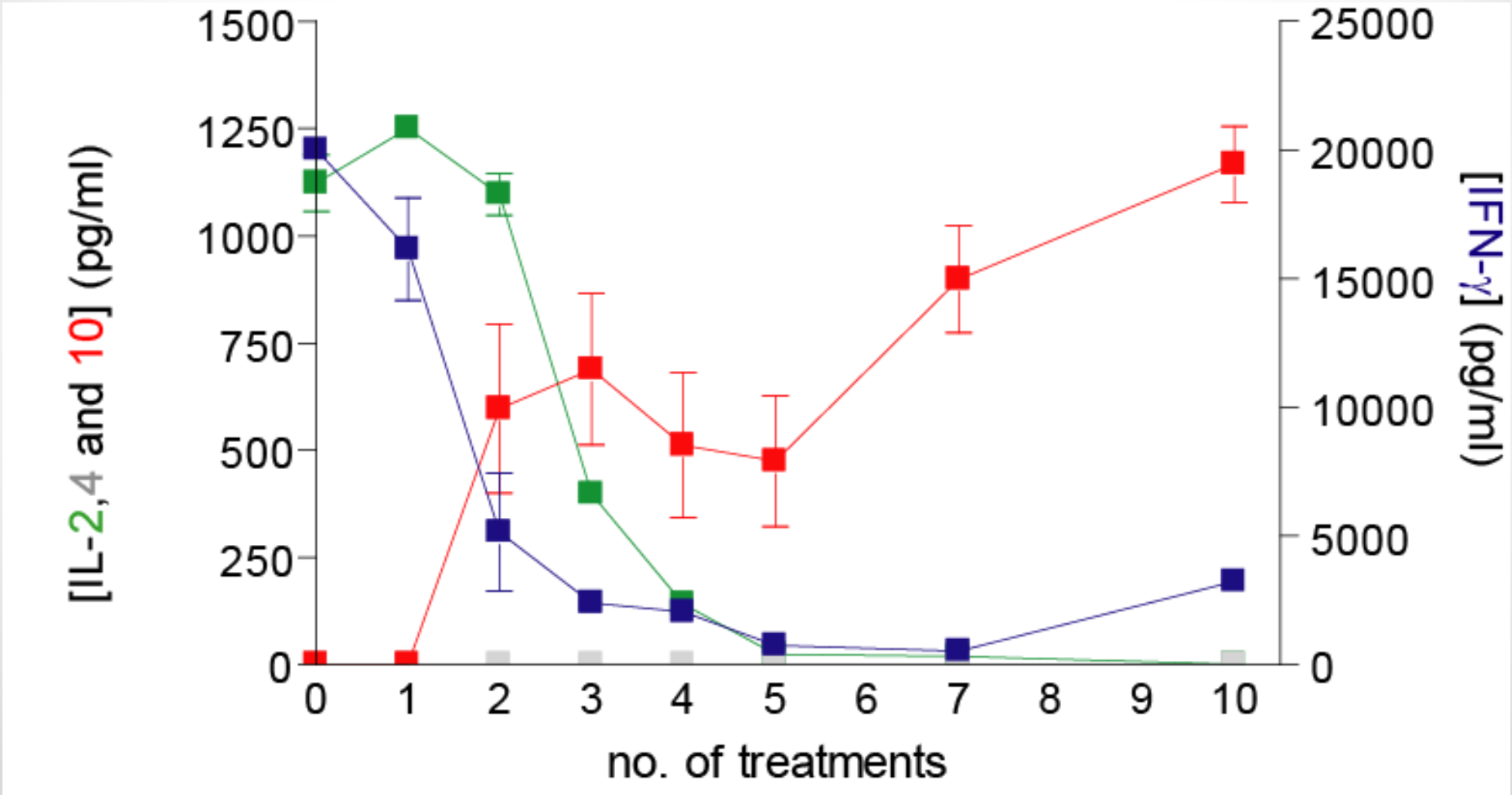
Kinetic studies: induction of anergy



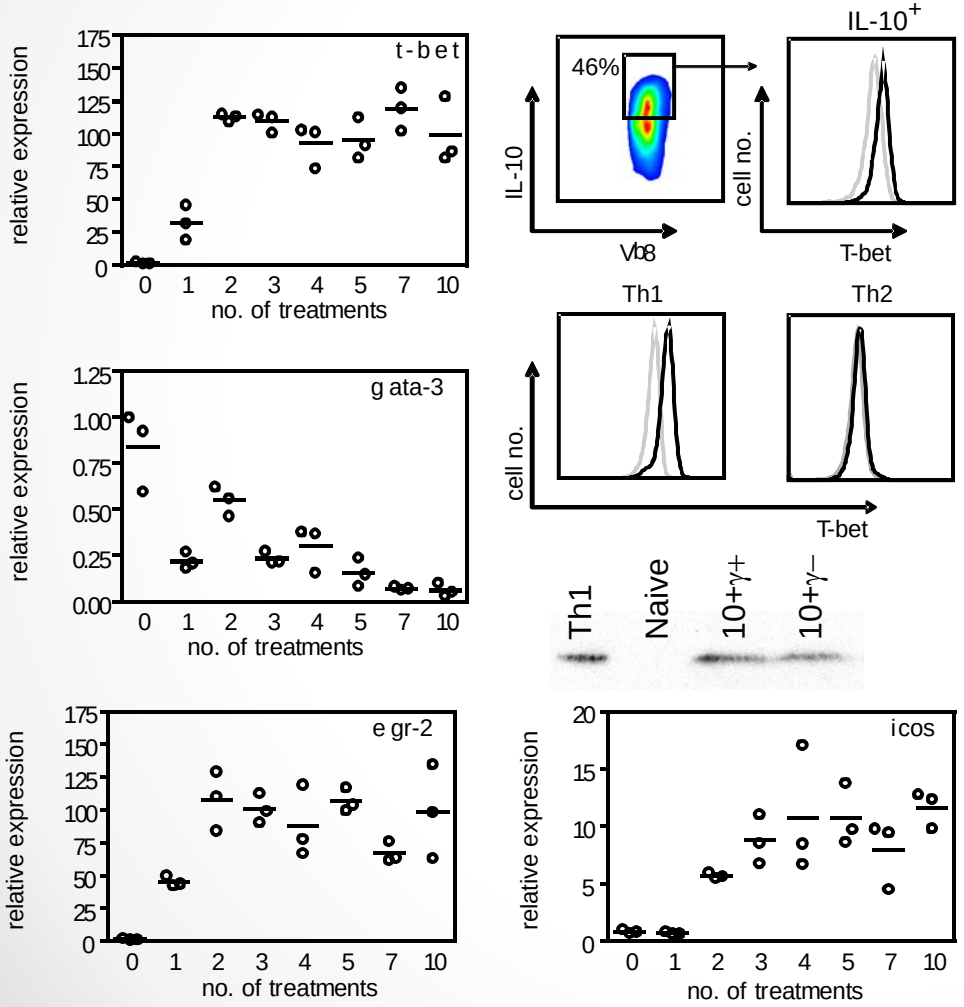
Tg4 mouse treated with peptide every 3rd or 4th day

Gabryšová et al: J. Exp. Med. (2009) 206: 1755-1767

Switch in serum cytokines

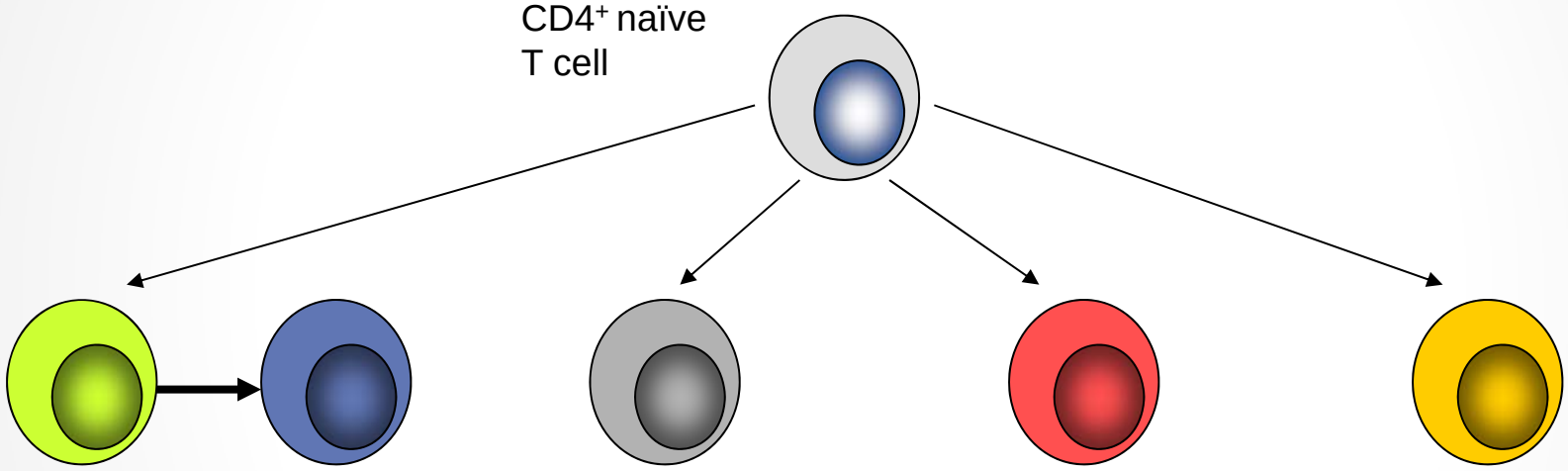


T-bet expression

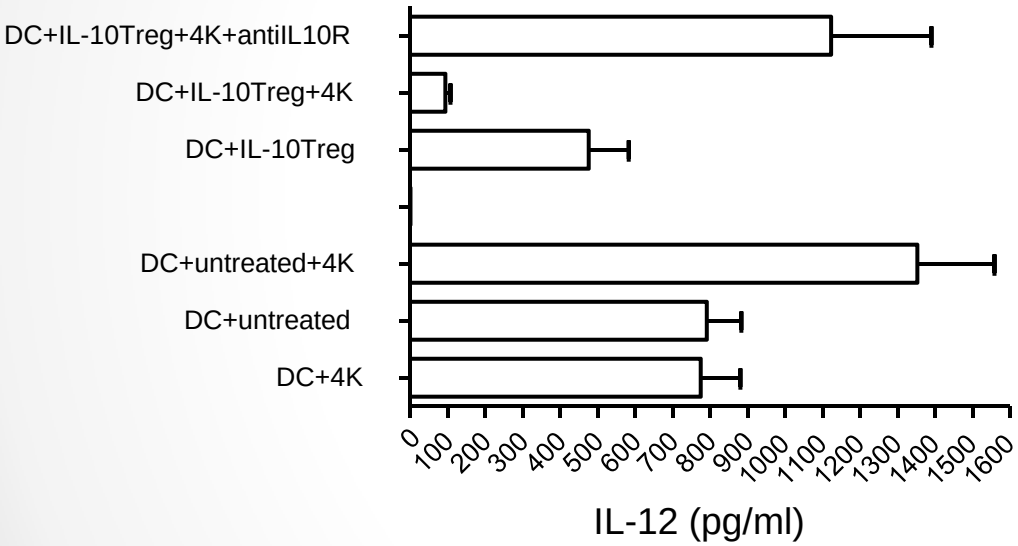


- T-bet is strongly upregulated in CD4$^+$ T cells
- EGR-2, an anergy associated gene, is strongly upregulated
- Purified IL-10 secreting cells express T-bet

Cytokines act on innate and/or adaptive immune cells to control the effector response



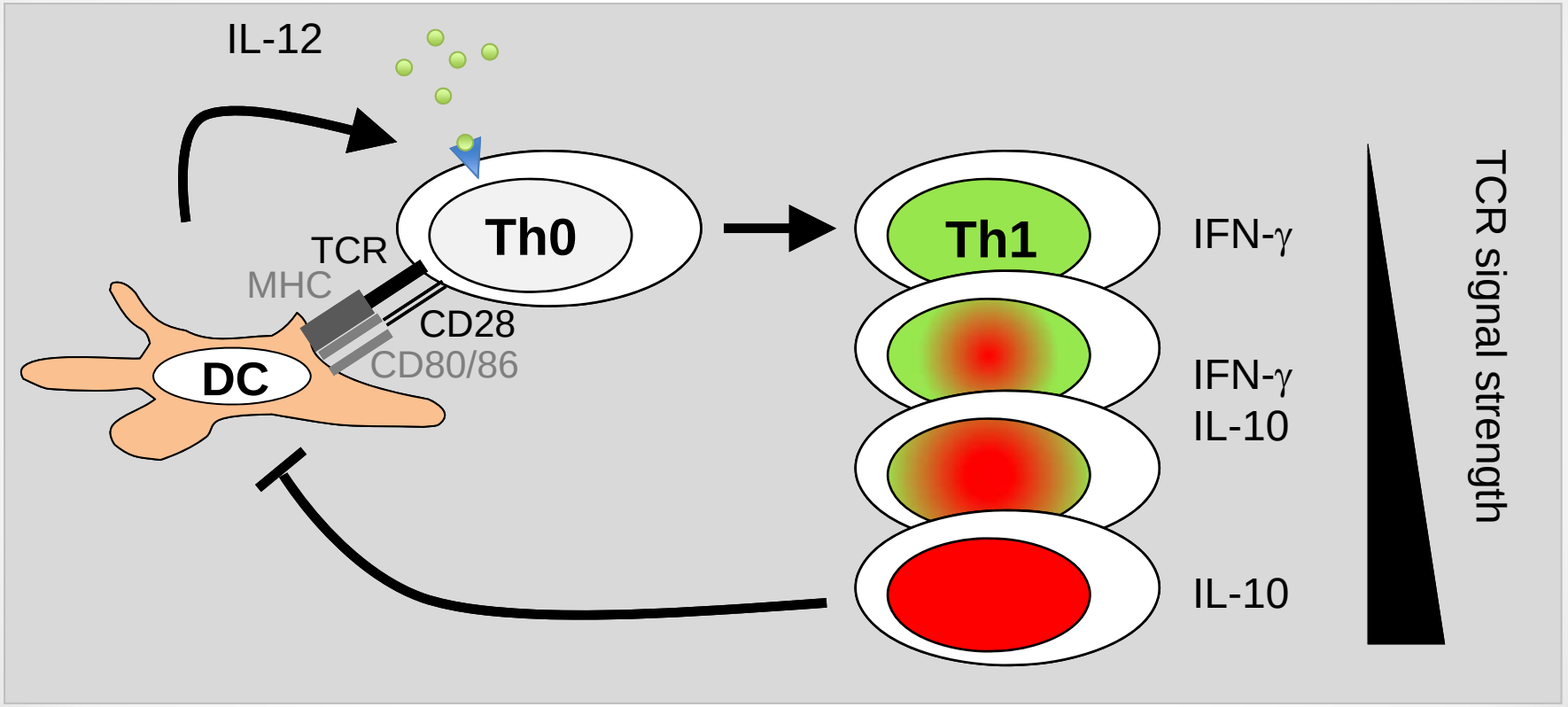
Role of IL-10



- Cognate interaction between T cells and DC leads to upregulation of IL-12 secretion
- IL-10 Treg cells inhibit IL-12 secretion by DC
- Inhibition of IL-12 secretion is IL-10 dependent

Gabryšová et al: J. Exp. Med. (2009) 206: 1755-1767

Negative feedback of the Th1 response: signal strength



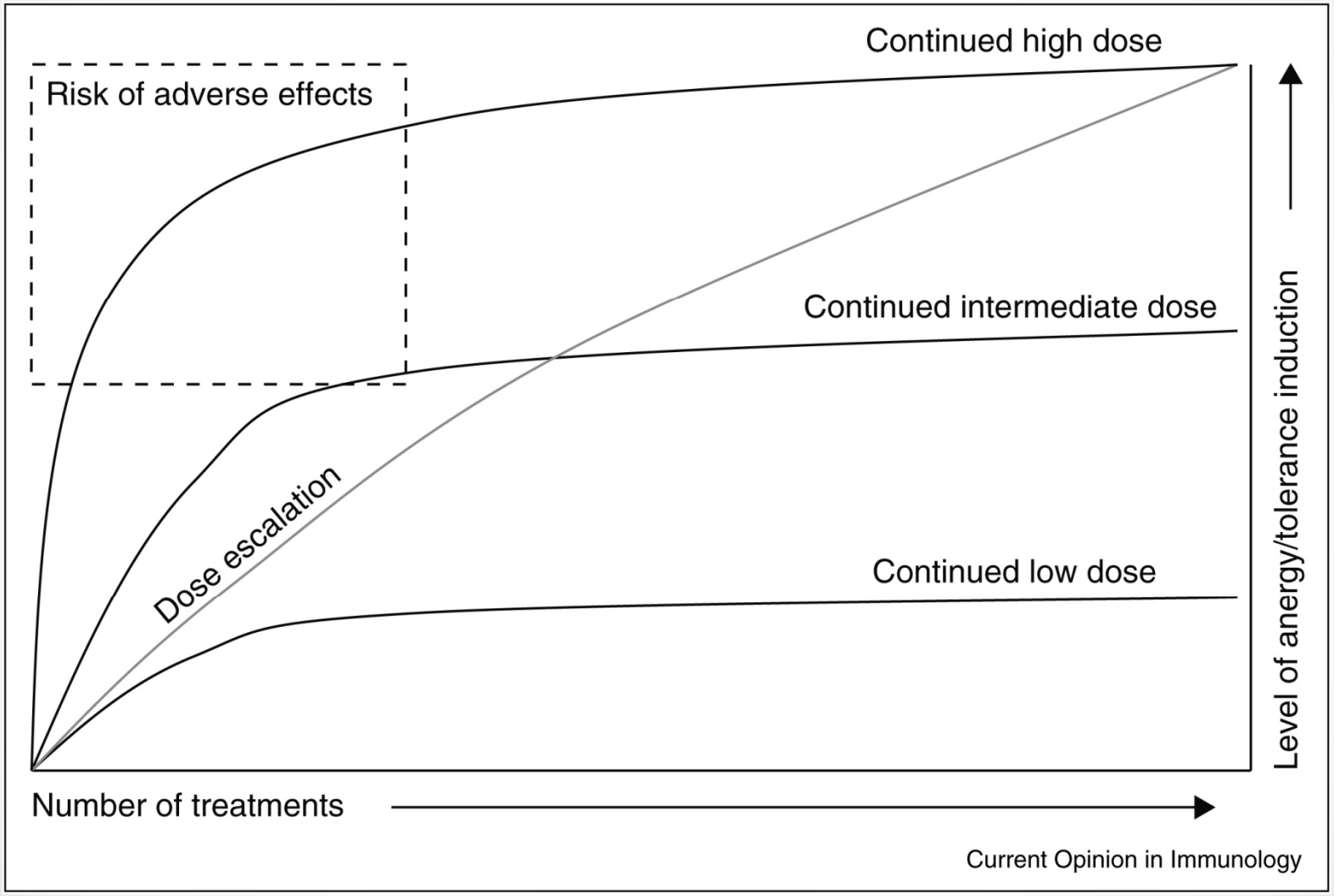
Gabryšová & Wraith Eur. J. Immunol. (2010) 40: 1386-1395

Route of administration

- The intranasal route of peptide administration has proven safe and effective for induction of IL-10 secretion and tolerance
- The equivalent high dose of peptide induces high cytokine levels after the third dose (female >> male) in Tg4 but not in non-transgenic mice, when given subcutaneously
- A 100-1000x lower dose of peptide induces tolerance via the s.c. route when given repeatedly

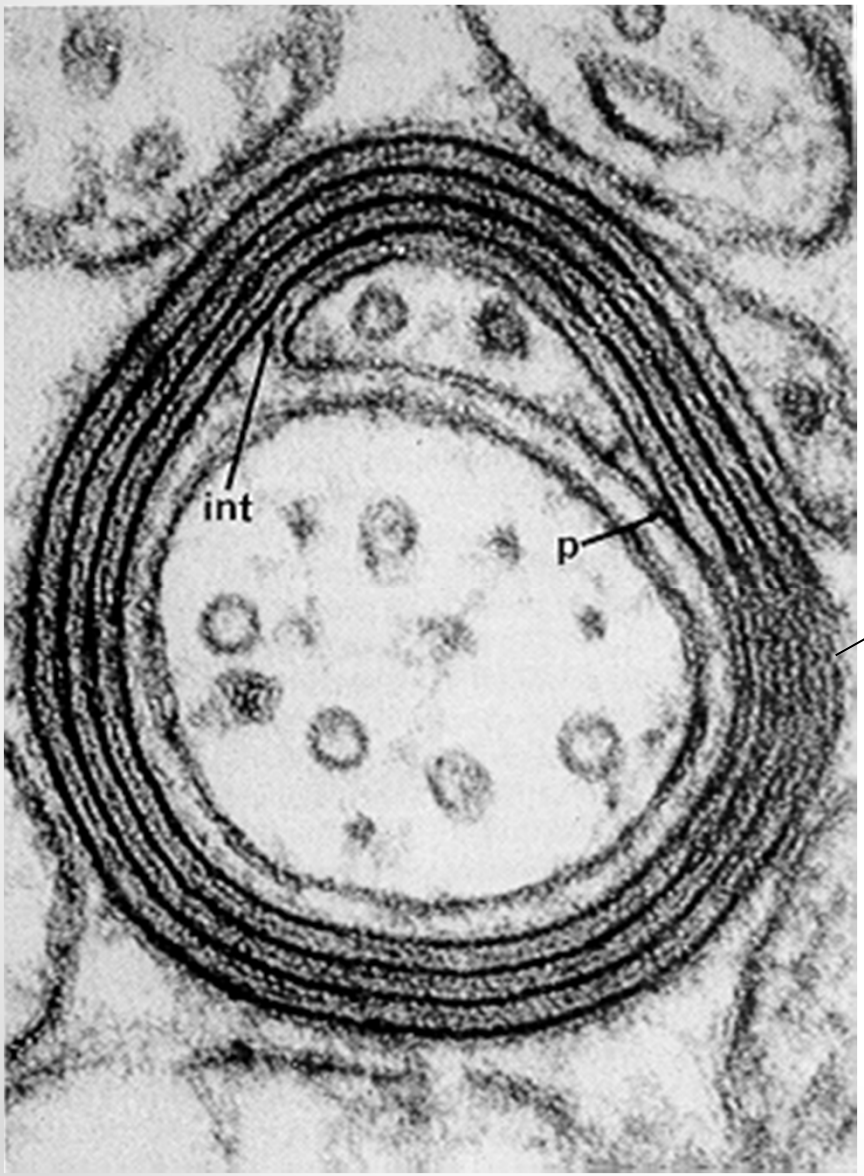
Bronwen Burton: unpublished

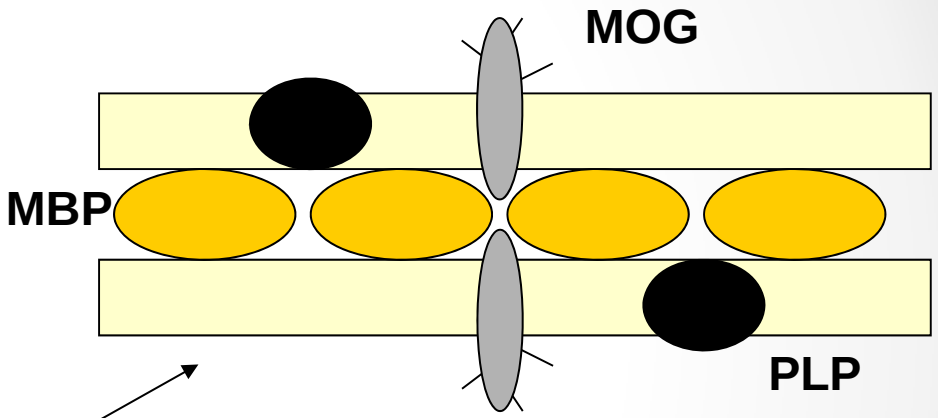
Rationale for dose escalation



Sabatos-Peyton, C., Verhagen, J. & Wraith, D.C. Antigen-specific immunotherapy of autoimmune and allergic diseases. Current Opinion in Immunology (2010) 22: 609-615

Peptides therapy in MS





R. O. Weller

Epitopes in MBP

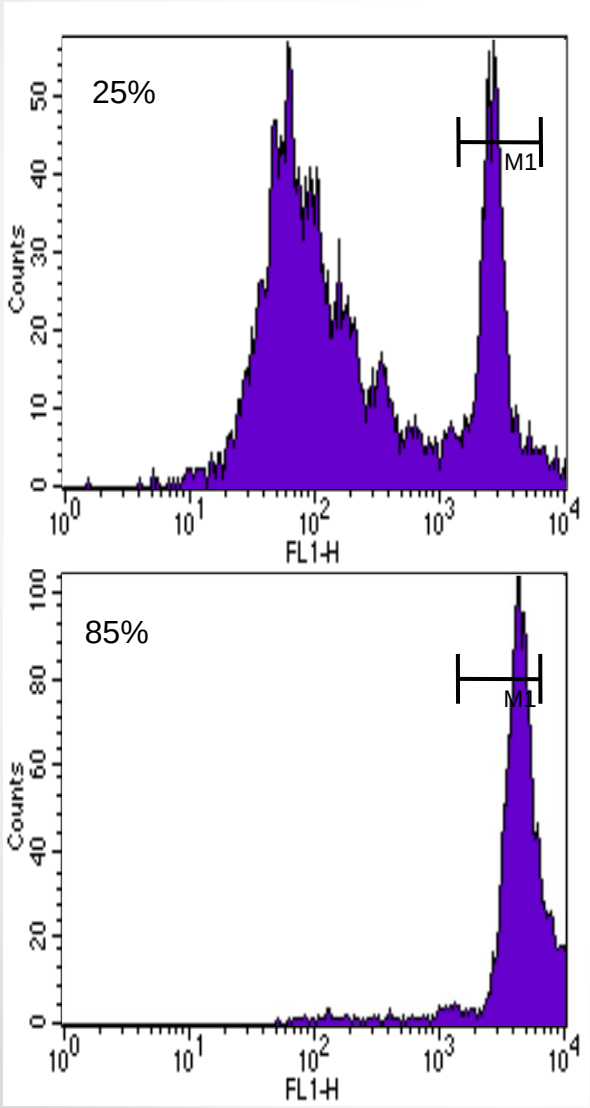
MS 1 30-44 DQ 6	MS 7 83-99 DR 15	MS 4 131-145 DQ 6	MS 6 140-154 DR 15

MS1467

Pre-clinical testing of MS apitopes in humanised mouse model

- Mice express HLA-DR15 and human TCR specific for MBP
- Demyelinating inflammation can be induced by immunisation with myelin, myelin proteins or peptides
- Treatment with ATX-MS1467 prevents disease and suppresses ongoing disease

ATX-MS1467: mechanism of action



PBS treated

ATX-MS1467 treated

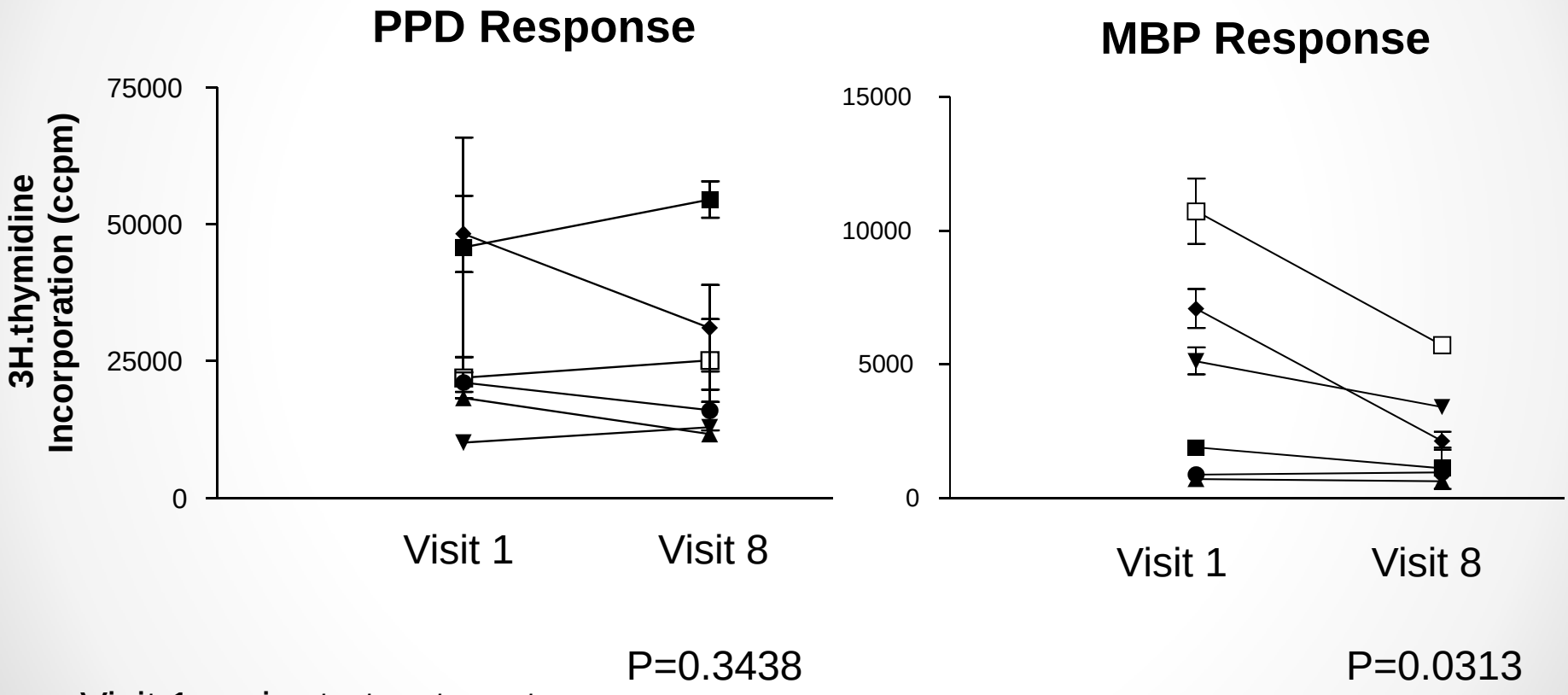
- ATX-MS1467 peptide treatment leads to the induction of anergy
- Treatment suppresses secretion of inflammatory cytokines (IL-1α, IL-2, IL-6, IL-17, IFN-γ, TNF-α, GMSCF)
- Secretion of anti-inflammatory cytokine (IL-10) sustained

Phase I: protocol

- **Subjects**
  - Patients (6) with secondary progressive multiple sclerosis (SPMS)
- **Design:**
  - Open-label
  - Dose-escalation of five doses, plus repeat of highest dose
- **Posology**
  - Dose frequency: 7 to 14 days apart
  - Dose Escalation: 25, 50, 100, 400, 800, 800 μg i.d.
- **Primary objective**
  - Assess safety and tolerability of ATX-MS-1467
- **Secondary objective**
  - Monitor immunological parameters in response to ATX-MS-1467
  - Monitor disease status in the CNS using MRI

Subject No.	HLA DR Type	Disease score Start (V1)	Disease score End (V9)	Clinical observations
P2	DRB1*01; DRB1*11	5.0	5.0	
P4	DRB1*11; DRB1*15	6.5	6.5	
P5	DRB1*04 DRB1*04	6.5	6.5	
P6	DRB1*13; DRB1*14	7.5	7.5	Improvement in vision Right eye: 6/24 (V1) to 6/9 (V9) Left eye : 6/9 (V1) to 6/6 (V9)
P7	DRB1*13; DRB1*13	6.0	6.0	Improvement in Gd-enhanced MRI on Visit 8 (1 month follow-up)
P8	DRB1*01; DRB1*07	7.5	7.5	

Immune responses

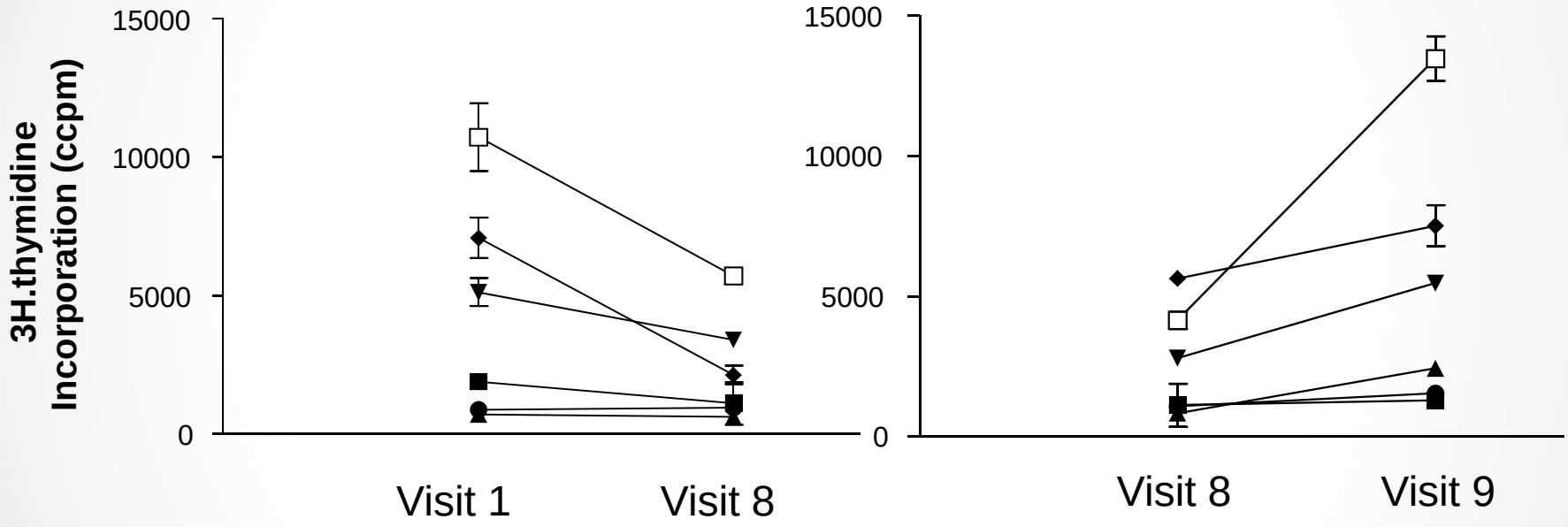


P=0.3438

P=0.0313

Visit 1: prior to treatment
Visit 8: one month after last dose

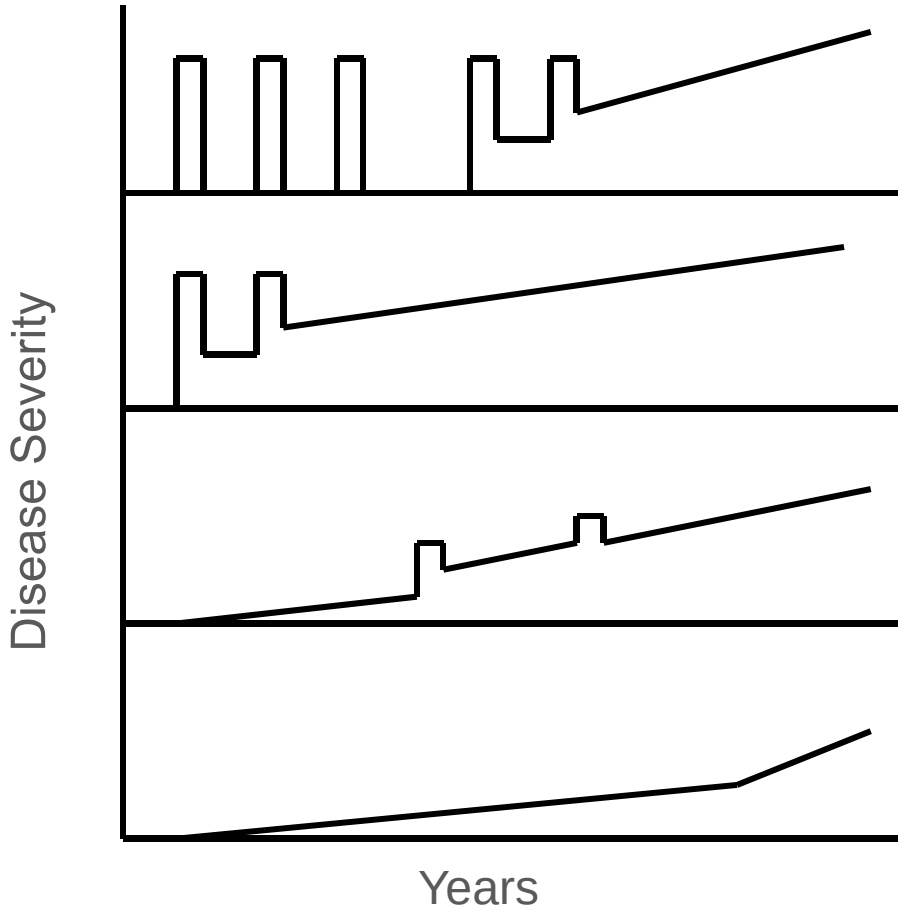
Immune responses



Visit 1: prior to treatment
Visit 8: one month after last dose
Visit 9: three months after last dose

Repeated treatment with ATX-MS-1467 will be required to maintain suppression of the immune response to myelin antigen

Patterns of MS



- A further trial comparing ID and SC routes of ATX-MS1467 is in progress
- This second trial is recruiting patients with relapsing multiple sclerosis

ClinicalTrials.gov Identifier: NCT01097668 Dr Jeremy Chataway

Allergy

Published June 15, 2009

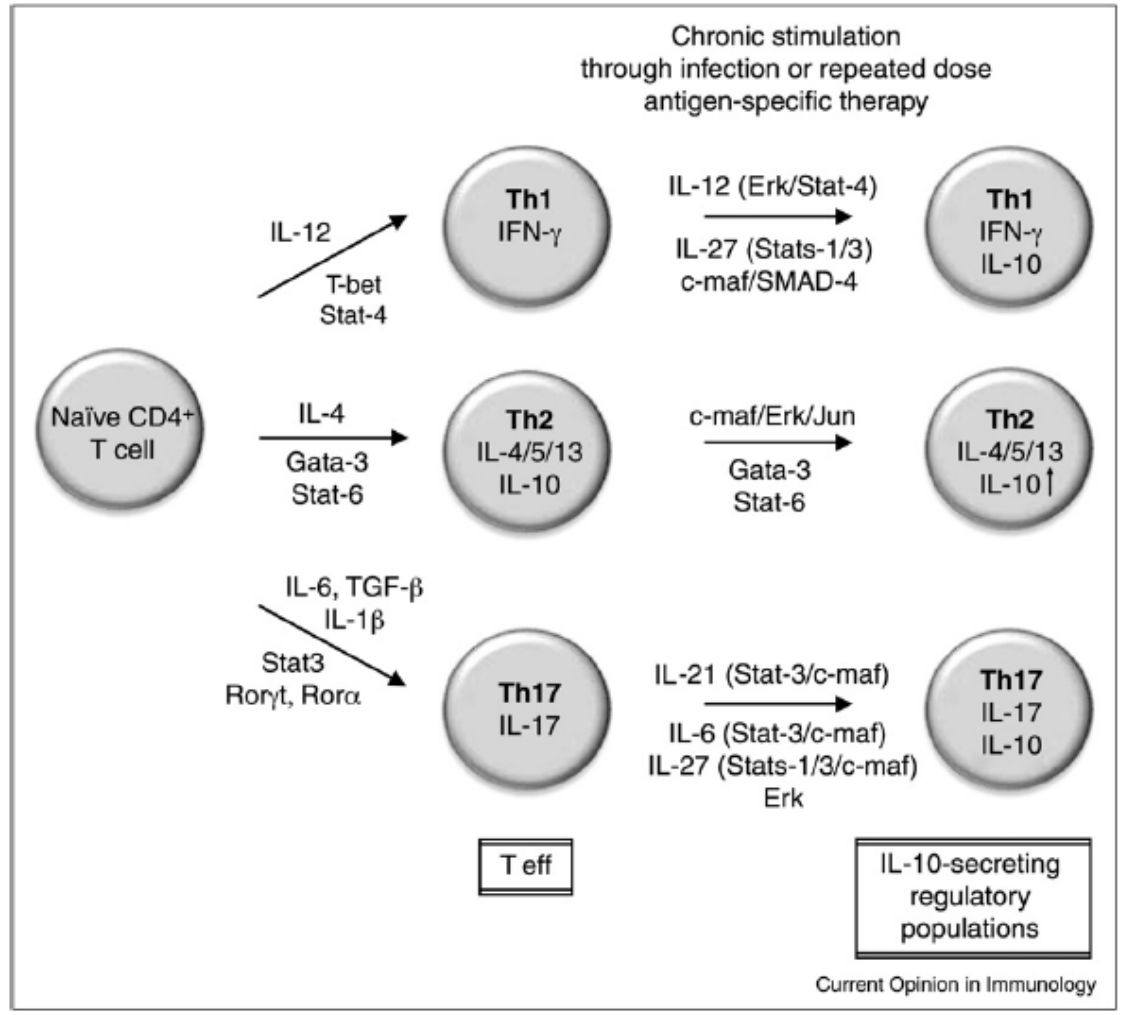
JEM

ARTICLE

Peptide immunotherapy in allergic asthma generates IL-10–dependent immunological tolerance associated with linked epitope suppression

John D. Campbell,[1,2] Karen F. Buckland,[1,2] Sarah J. McMillan,[1,2] Jennifer Kearley,[1,2] William L.G. Oldfield,[4] Lawrence J. Stern,[6] Hans Grönlund,[7] Marianne van Hage,[7] Catherine J. Reynolds,[1,3,4] Rosemary J. Boyton,[1,3,4] Stephen P. Cobbold,[8] A. Barry Kay,[1,2,4] Daniel M. Altmann,[5] Clare M. Lloyd,[1,2] and Mark Larché[1,4,9]

IL-10 and negative feedback mechanisms



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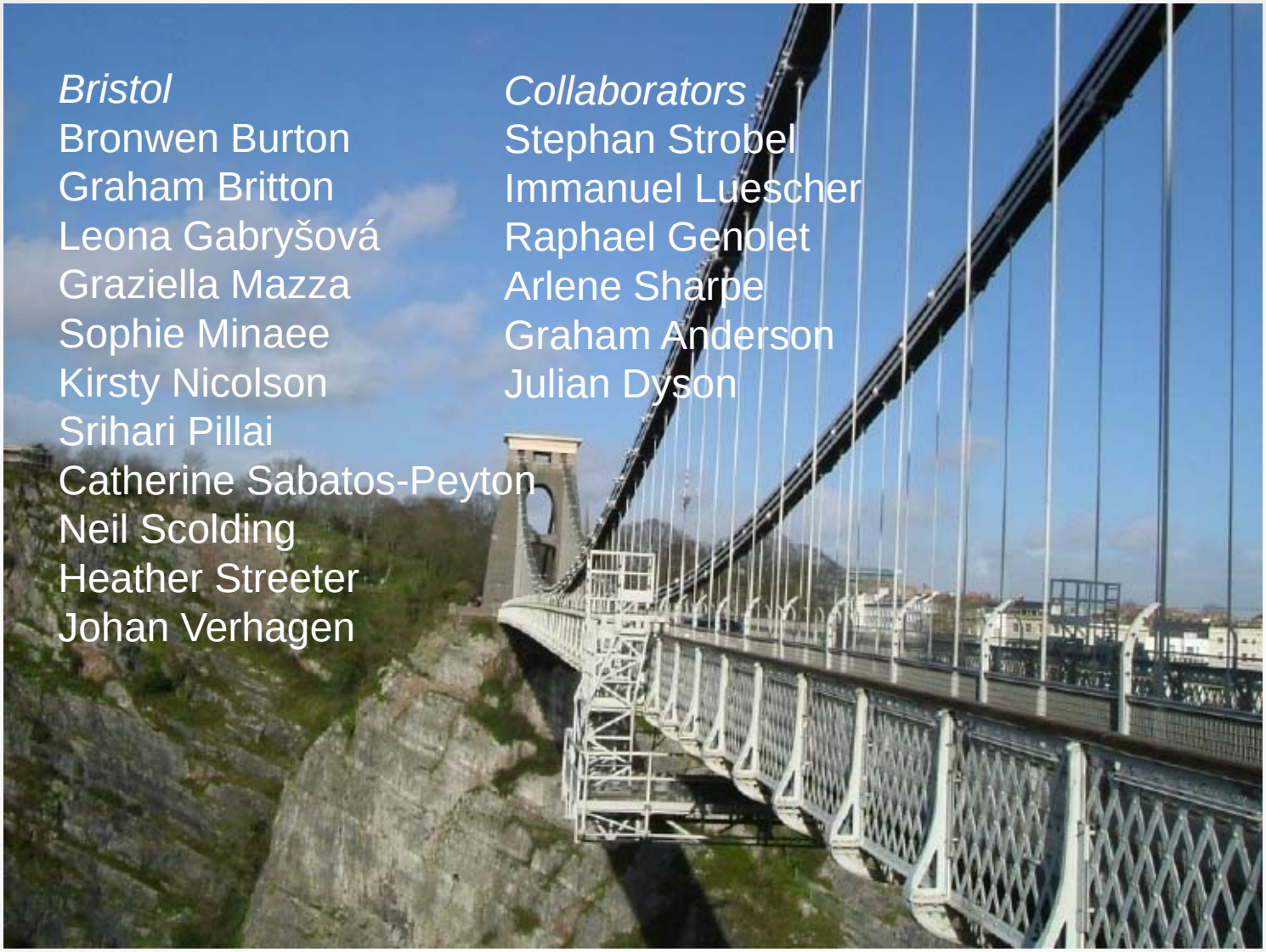

Our laboratory is supported by the Wellcome Trust and the Multiple Sclerosis Society of Great Britain and Northern Ireland. The phase I/IIA clinical trial in MS was funded by a Wellcome Trust UTA to Apitope