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Beyond the next generation: data mining and T cells

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Overview

Data mining

- Getting the most from diagnostic data

The host immune response to *Borrelia*

- Potential targets for new diagnostic tests

T cell tests for Lyme diagnosis

New diagnostic targets

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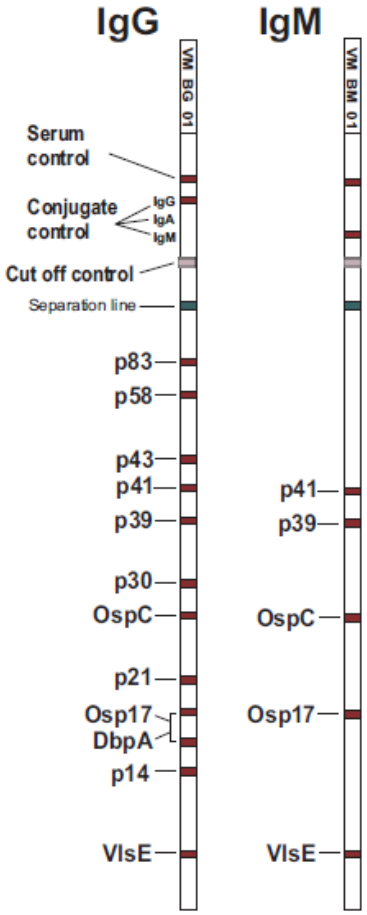
Getting the most out of diagnostic results



Current analysis of ViraStripe immunoblots

For IgM blot:
Positive result if 1 of 5 bands is > cut-off threshold*

For IgG blot:
Positive results if 3 of 12 bands are > cut-off threshold*

*Band cut-offs are determined on each blot automatically, but are *c.*80 for all bands except p41 which is around 150.

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What else is hidden in the data?

	DATE	01/2013	08/2012	07/2012	06/2012
IgG blots	p83	-	41	-	-
	p58	-	-	-	-
	p43	-	-	-	-
	p41	-	57	41	66
	P39 (BmpA)	-	-	-	-
	p30	-	-	-	-
	OspC	-	-	-	-
	p21	-	-	-	-
	Osp17	-	-	-	-
	DbpA	40	26	45	86 (POS)
	p14	-	-	-	-
	VlsE	51	44	-	-
IgM blots	p41	-	65	-	125
	p39	-	31	-	-
	OspC	145 (POS)	128 (POS)	133 (POS)	300 (POS)
	Osp17 (OspA)	-	-	-	53
	VlsE	-	96 (POS)	-	
C6 ELISA	IgM / IgG combined OD	4.001	4.444	5.338	5.892 (POS)

Patterns of bands and the relative density of bands may reflect, for example:

- Disease progression over time
  - Serial samples
- Disease stage
  - Single samples
- Species of infecting *Borrelia*

→ Data mining

Compare band patterns and densities with clinical and diagnostic data

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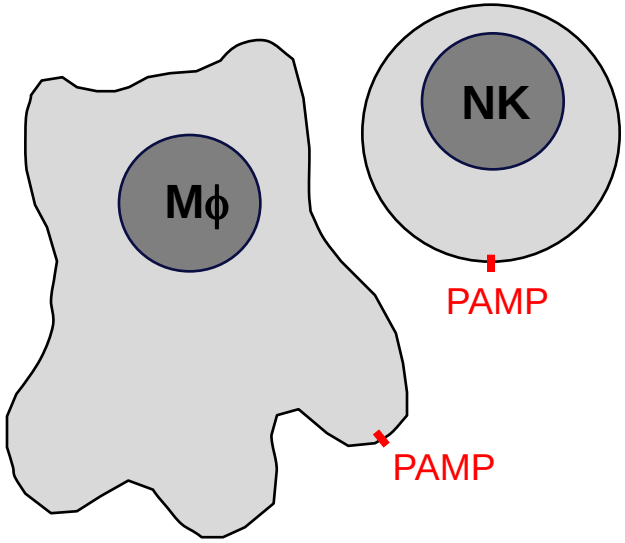
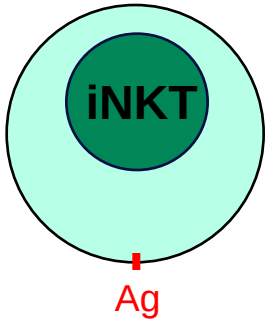
Players in the host immune response to *Borrelia*

Innate Immune Response

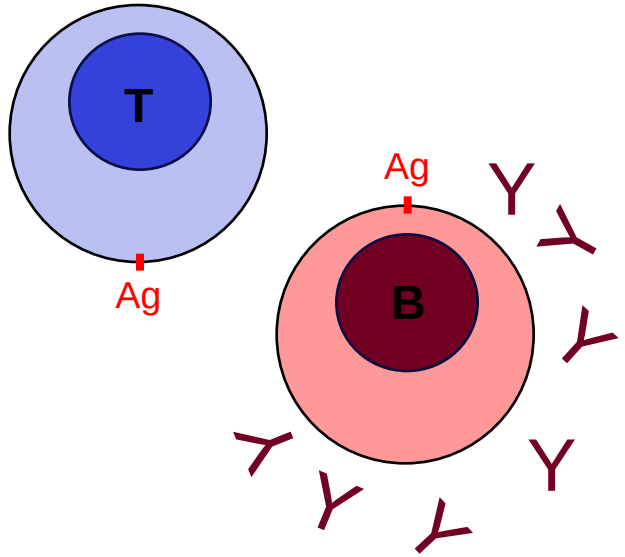


Non-specific
Rapid
Recognises common pathogen associated molecular patterns (PAMPs)



Moderate specificity
Rapid
Recognises glycolipid antigens

Adaptive Immune Response



Specific
Slow
Recognises individual *Borrelia*-derived antigens

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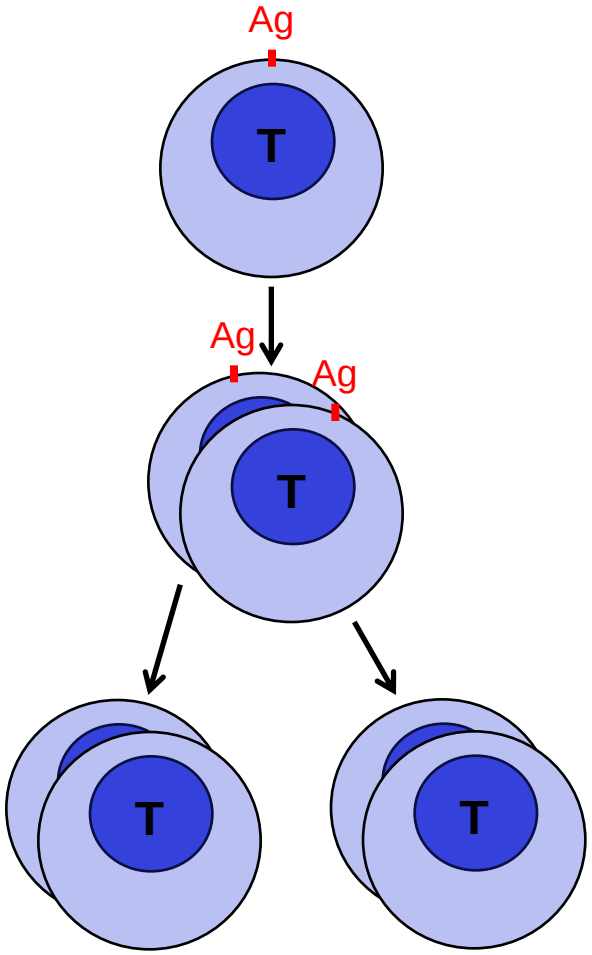
T cells as targets for diagnostic tests (1)

T cell proliferation

- T cell activated with its specific *Borrelia* antigen divides and proliferates
- Measured *in vitro* by incorporation of labelled nucleotide e.g. 3H-thymidine, into dividing DNA
- Assays typically use PBMCs
- Various formats/names:
  - Lymphocyte Transformation Test (LTT)
  - Lymphocyte Transformation Assay (LTA)
  - T cell proliferation assay
  - LTT-MELISA

Widely used in Lyme disease **research**



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T cell proliferation as a Lyme diagnostic tool?

•Investigated as **diagnostic tool** in over 20 peer-reviewed scientific publications

•No consensus on value of LTT as diagnostic test

- equivalent levels of proliferation in normal donors and Lyme patients (=poor specificity)
- negative LTT/LTA in clinically & serologically positive Lyme patients (=poor sensitivity)
- Proposed role for monitoring treatment course and to confirm successful therapy
- Proposed role to identify early active disease in seronegative patients i.e. before Ab produced.

- Current state-of-the-art: ONLY run LTT/LTA test with other tests. Beware over-interpretation of results.

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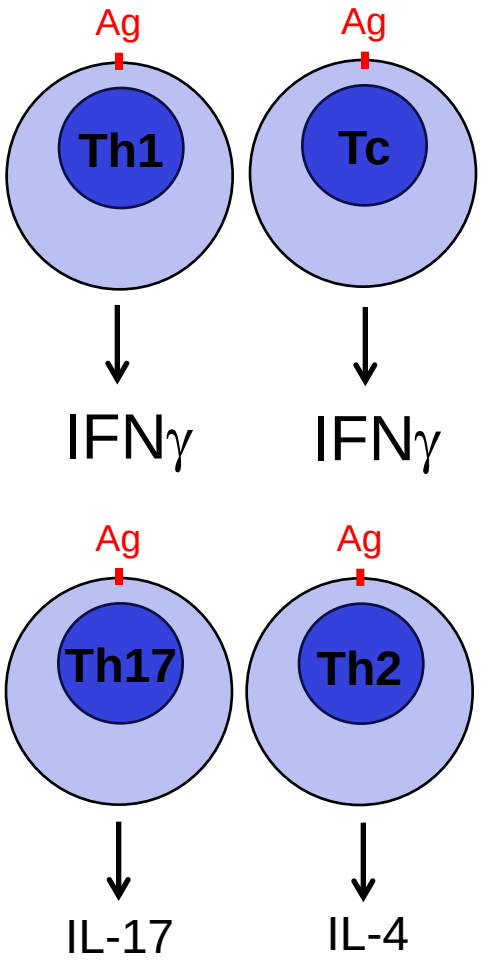
T cells as targets for diagnostic tests (2)

T cell cytokine production

- T cell activated with its specific *Borrelia* antigen produces and releases cytokines
- Cytokine released depends on type of *Borrelia*-specific T cell (Tc, Th1, Th2, Th17 etc)
- Released cytokine measured *in vitro* by:
  - ELISPOT / immunospot / iSpot Lyme™
  - IFNγ-release assay (IGRA)
  - ELISA
  - Luminex/ bead-based assay



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IFNγ ELISPOT as a Lyme diagnostic tool?

- Used in Lyme disease **research** since late 1990s
- More rapid (overnight) than LTT (3-5 days)
- Investigated as **diagnostic tool** in few peer-reviewed scientific publications
- No consensus on value of IFNγ ELISPOT as diagnostic test for Lyme
  - Not suitable as supplementary diagnostic tool in blood (Forsberg et al 1995)
  - Not suitable as supplementary diagnostic tool in CSF (Nordberg et al 2012)
- Diagnostic value of ELISPOT for other cytokines e.g. IL-17 & IL-4 not tested

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Improving LTT & ELISPOT assays

Rely on living cells (whole blood or PBMCs)

- standardise transport and storage (times, temperature etc)
- standardise input cell numbers

Source of antigen: whole *Borrelia,* lysates or single antigens?

- glycolipids and lipopeptides in whole *Borrelia* and lysates induce high background levels of proliferation; batch-to-batch inconsistency of whole *Borrelia*
- Newer assays are using defined *Borrelia* Ags
- Ensure relevant *Borrelia* species (USA *versus* Europe)

Need for detailed longitudinal clinical studies

- compare T cell-based and other diagnostic tests (Ag & Ab)

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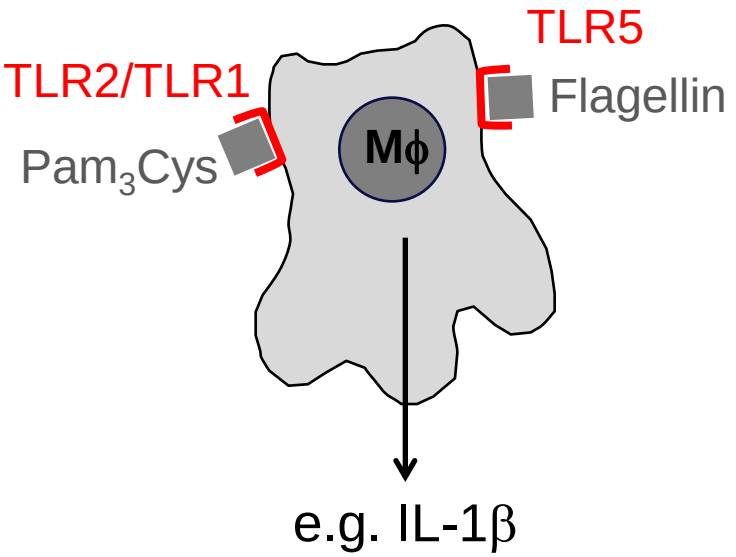
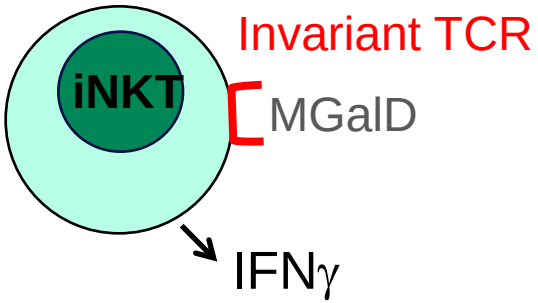
New diagnostic possibilities

- TLR2/TLR1 heterodimers recognise acylated borrelial Osp lipoproteins
- diacylated & triacylated
- Basis of SpiroFind™ assay under development and testing by Boulder Diagnostics
- *Borrelia* cocktail used for stimulation
- Potential for false positives





- Invariant NKT cells recognise low molecular weight glycolipids on *Borrelia* surface e.g. MGalD
  - proliferate & release IFNγ
- May be possible to mimic *in vitro* using synthetic glycolipids

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Summary

- New generation, standardised T cell assays need to be developed and applied in longitudinal clinical studies
- Diagnostic tests based on the innate and immediate immune response warrant further detailed investigation
- Results from new tests need to be compared with current diagnostic tests **in the same studies**
- New diagnostic tests should not be limited to one specialist commercial lab; need external validation and analysis of reproducibility
- Data mining expertise will maximise the predictive power of current and future diagnostic tests

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Lyme disease conference

9 October 2013