

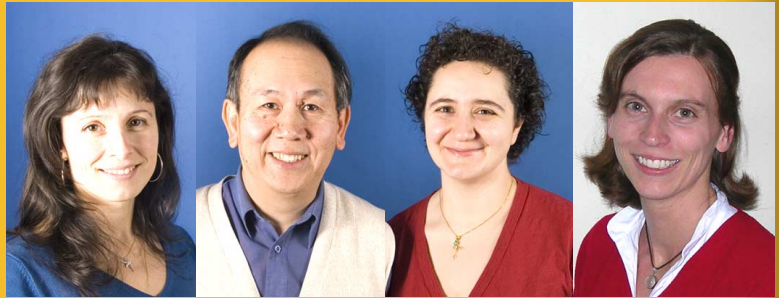
## INTERVIEW about

## Targeting antigen to dendritic cells

with

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From left: Irina Caminschi, Andrew M Lew, Mireille Lahoud, Alexandra J Corbett.



### How many targeting receptors on dendritic cells are known?

**Irina Caminschi, Alexandra J Corbett, Mireille Lahoud & Andrew M Lew:** The Table summarizes the numbers of molecules on dendritic cells (DC) that have been targeted *in vivo* and the resultant outcomes. The majority have resulted in enhancing immune responses but there are examples of “tolerizing” responses e.g. targeting 33D1 and CD205.

### Are there differences between CD8 positive and CD8 negative DC subsets?

**Irina Caminschi, Alexandra J Corbett, Mireille Lahoud & Andrew M Lew:** There are plasmacytoid DC (pDC) whose steady state morphology is similar to a plasma cell (i.e. not dendritic) and are characterized by medium expression of CD11c and high expression of CD45RA. Unless otherwise stated, the term DC refers to “conventional” DC (cDC), defined as expressing high levels of the integrin CD11c, and MHC class II and are potent antigen presenting cells capable of activating naïve T cells. However, cDC are heterogeneous so in the spleen for example, they have been classified into three groups according to their surface markers: CD4<sup>+</sup>8<sup>+</sup> (CD4<sup>+</sup>), CD4<sup>+</sup>8<sup>-</sup> (CD8<sup>+</sup>), CD4<sup>-</sup>8<sup>-</sup> (DN) (1, 2). These classifications correlate with biological activities. From a functional perspective, the greatest differences are seen between the CD8<sup>+</sup> DC and CD4<sup>+</sup> or DN DC (collectively referred to as CD8<sup>-</sup> DC). CD8<sup>+</sup> DC are the most potent producers of IL-12p70 (1) and therefore direct a T helper 1 (Th1) cytokine production profile whereas CD8<sup>-</sup> DC promote a Th2 response (3,4). CD8<sup>+</sup> DC constitutively ‘cross-present’: a CD8<sup>+</sup> DC can present antigen that is not synthesized by the CD8<sup>+</sup> DC itself. CD8<sup>+</sup> DC can present soluble or cell-associated exogenous antigens on class I MHC and thereby activate CD8<sup>+</sup> T cells (5,6). At least in some cases, they have been shown to be major presenters of viral antigens (7,8). By contrast, CD8<sup>-</sup> DC generally do not cross-present antigen but are more efficient than the CD8<sup>+</sup> DC at MHC class II-restricted presentation to CD4<sup>+</sup> T cells (5). Interestingly, whereas in the activated state, CD8<sup>+</sup> DC are effective inducers of CD8<sup>+</sup> T cell responses, in the quiescent state they have special regulatory mechanisms to help maintain self-tolerance. For example, CD8<sup>+</sup> DC suppress delayed type hypersensitivity reactions (9) and in the draining lymph nodes they induce deletional T cell tolerance to a model pancreatic self-antigen (10). In agreement with this, *in vitro* CD8<sup>+</sup> DC are less stimulatory than CD8<sup>-</sup> cDC, both in terms of T cell activation and induction of cytokine production (11-15). Thus, CD8<sup>+</sup> DCs have been proposed to be tolerogenic when presenting innocuous self-antigens, but immunogenic when antigen is detected in the context of danger/inflammatory signals such as microbial substances. DC can recognize an array of microbial components utilizing

a group of Toll-like receptors (TLR). All cDC express TLR 1, 2, 4, 6, 8, and 9, but only CD8<sup>-</sup> DC express TLR 7, and the CD8<sup>+</sup> DC preferentially express TLR 3 (16). Effectively this

means that different DC subtypes are activated by different microbial products (17) and therefore can differ in their responses (18) and their ability to direct immune responses.

### What is the difference between FIRE and CIRE targeting and CD205?

**Irina Caminschi, Alexandra J Corbett, Mireille Lahoud & Andrew M Lew:** A great deal of work has been done on characterizing the immune response elicited by targeting DC using CD205, a marker expressed on CD8<sup>+</sup> DC. Essentially, to generate a potent immune response, anti-CD205 mAb is co-administered with an activation or maturation stimulus such as anti-CD40 mAb or LPS. Targeting CD205 without such an adjuvant results in an initial T cell response, which is followed by deletional tolerance (19,20). In accordance with these observations, mice immunized with rat anti-CD205 mAb in the absence of adjuvant, did not induce anti-rat serum Ig responses (21). In stark contrast, immunization with anti-CIRE or anti-FIRE rat mAb provoked potent Ab production, which was up to 1000-fold higher than a non-targeted control, and importantly, this was achieved without the need for any adjuvants (21). How can such difference in humoral immunity be reconciled? One possibility was that our mAbs against FIRE and CIRE had been contam-

inated with LPS, which could act as a maturation/activation signal. We quantitated the levels of endotoxin in our mAb preparations and determined that the minute levels present were insufficient to act as adjuvants. This was tested using our non-targeting mAb isotype control.

Hence, the difference between targeting with CD205 and FIRE/CIRE appears to be based on other variables. CD205 is expressed on CD8<sup>+</sup> DC, the subset of DC that have been implicated in immunity as well as peripheral tolerance; by contrast, CIRE and FIRE are expressed on CD8<sup>-</sup> DC. It is conceivable that targeting to CD8<sup>+</sup> DC without a danger signal defaults to tolerance rather than immunity, and perhaps the same rule does not apply when targeting CD8<sup>-</sup> DC. Another factor that needs to be considered is the molecules themselves; it may be possible that each molecule impacts on the fate of the antigens that attach via mAbs. Future work will aim to delineate the role of the molecules versus the role of the DC subsets targeted using mAbs.

## Summary of DC-targeting strategies in vivo

Target molecule	Cells targeted	Targeting strategy	B cell response	T cell response	↑ Protection	References
33D1	Splenic DC	Rat mAb 33D1 low dose Anti-CD11c + anti-33D1	Tolerance ↑ Ab	Tolerance		(22)
CD80/86	DCs, B cells, macrophages,	CTLA4-Ig DNA	↑ Ab Accelerated Ab (mice, sheep)	↑ prolifer CTLs not changed	influenza in mice, C. pseudotuberculosis in sheep	(23-25)
CD11c	DC (immature)	Hamster mAb N418	↑ Ab			(26)
CD40	DC  B cells, epithelial cells	Bispecific Ab targeting adenovirus vector CD40L (bovine) DNA fusion to antigen	↑ Ab (sheep)		tumour	(27) (28)
CCR6 chemokine receptor	Immature DC	β-defensin 2 and 3 DNA	↑		tumour	(29)
CIRE	Immature CD8 <sup>+</sup> DC	Rat mAb 5H10	↑ Ab			(21)
Complement receptors CR1/2	B cells, Follicular DCs	Rat mAb 7G6	↑ IgG1			(30)
		C3d fusion	↑ Ab			(31)
CD205	CD8 <sup>+</sup> DC	Rat mAb NLDC-145		↑ prolifer early, then unresponsiveness		(19)
		OVA conjugated Ab		↑ prolifer early, then unresponsiveness		(20)
FcγR1	DCs, macrophages	Ab	↑	↑ CD4 <sup>+</sup> and CD8 <sup>+</sup> T ↑ CTLs		(32, 33)
FIRE	Immature CD8 <sup>+</sup> DC, monocytes, macrophages	Rat mAb 6F12	↑ Ab			(21)
Glycolipid Gb3	DCs	Shiga toxin B subunit		↑ CTLs		(34)
MHC class II	B cells, DCs, macrophages	mAb-biotin	↑ to avidin			(35)
		mAb-Ag conjugates + cholera toxin or anti-CD40	↑ Ab			(36)
		Ab containing T cell epitopes		↑ CD4 <sup>+</sup>		(37)

## REFERENCES

- Hochrein H et al. J Immunol 166, 5448, 2001
- Vremec D et al. J Immunol 164, 2978, 2000
- Moser M et al. Nat Immunol 1, 199, 2000
- Pulendran B et al. Trends Immunol 22, 41, 2001
- Pooley J et al. J Immunol 166, 5327, 2001
- den Haan JM et al. J Exp Med 192, 1685, 2000
- Belz GT et al. J Immunol 172, 1996, 2004
- Smith CM et al. J Immunol 170, 4437, 2003
- Grohmann U et al. J Immunol 167, 708, 2001
- Belz GT et al. J Exp Med 196, 1099, 2002
- Kronin V et al. J Immunol 157, 3819, 1996
- Kronin V et al. Immunol Cell Biol 78, 214, 2000
- Kronin V et al. Int Immunol 12, 731, 2000
- Kronin V et al. Int Immunol 13, 465, 2001
- Suss G et al. J Exp Med 183, 1789, 1996
- Schulz O et al. Nature 433, 887, 2005
- Takeda K et al. Annu Rev Immunol 21, 335, 2003
- Proietto AI et al. Immunobiology 209, 163, 2004
- Hawiger D et al. J Exp Med 194, 769, 2001
- Bonifaz L et al. J Exp Med 196, 1627, 2002
- Corbett AJ et al. Eur J Immunol 35, 2815, 2005
- Finkelman FD et al. J Immunol 157, 1406, 1996
- Boyle JS et al. Nature 392, 408, 1998
- Deliyannis G et al. PNAS USA 97, 6676, 2000
- Chaplin PJ et al. Infect Immun 67, 6434, 1999
- Wang H et al. PNAS USA 97, 847, 2000
- Tillman BW et al. Cancer Res 60, 5456, 2000
- Manoj S et al. J Immunol 170, 989, 2003
- Biragyn A et al. J Immunol 167, 6644, 2001
- Baiu DC et al. J Immunol 162, 3125, 1999
- Dempsey PW et al. Science 271, 348, 1996
- Heijnen IA et al. J Clin Invest 97, 331, 1996
- Guyre PM et al. Cancer Immunol Immunother 45, 146, 1997
- Haicheur N et al. J Immunol 165, 3301, 2000
- Carayanniotis G et al. Nature 327, 59, 1987
- Wu JY et al. Infect Immun 69, 7679, 2001
- Lunde E et al. J Immunol 168, 2154, 2002