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The Journal of Immunology

$CD4^+CD28^{null}$ T Cells in Autoimmune Disease: Pathogenic Features and Decreased Susceptibility to Immunoregulation[1]

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Introduction

$CD4^{+}CD28^{null}$ T cells are highly differentiated effector memory T cells.

As a response to chronic stimulation CD4+ T cells lose CD28 expression on their surface.

In chronic inflammation TNF-α may downregulate CD28 gene expression.

PIEPER J (2014). Peripheral and site-specific $CD4^{+}CD28^{null}$ T cells from Rheumatoid Arthritis patients show distinct characteristics

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Introduction

$CD4^+CD28^{null}$ T cells…

- have lost expression of CD40L
- are highly proinflammatory (cytokine production & cytotoxicity)
- express a variety of killer Ig-like receptors
- are considered to be autoreactive T cells
- are independent of co-stimulation

An expansion of $CD4^+CD28^{null}$ T cells has been reported in autoimmune diseases, HIV- & CMV-infections and coronary disease

PIEPER J (2014). Peripheral and site-specific $CD4^+CD28^{null}$ T cells from Rheumatoid Arthritis patients show distinct characteristics

What role do $CD4^{+}CD28^{null}$ T cells play in the pathogenesis of RA & MS?

Do $CD4^{+}CD28^{null}$ T cells display a reactivity towards foreign or autoantigens in RA & MS?

Can $CD4^{+}CD28^{null}$ T cells infiltrate into tissue and evolve their potential?

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Methods

Study population

4 multiple sclerosis patients

4 reactive arthritis patients

4 healthy control patients

peripheral blood for Ag reactivity and Ab reactivity in plasma against CMV

additionally: six paired blood-synovial fluid samples from reactive arthritis patients (PBMCs and SF mononuclear cells) + 2 synovial tissue samples

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Methods

FACS analysis

Isolation of CFSE labelling of sorted T cells

CFSE-based proliferation assay

Analysis of TCR BV gene usage and CD3R fragment length of CMV-reactive $CD4^{+}CD28^{null}$ T cells

Suppression assay

ELISA

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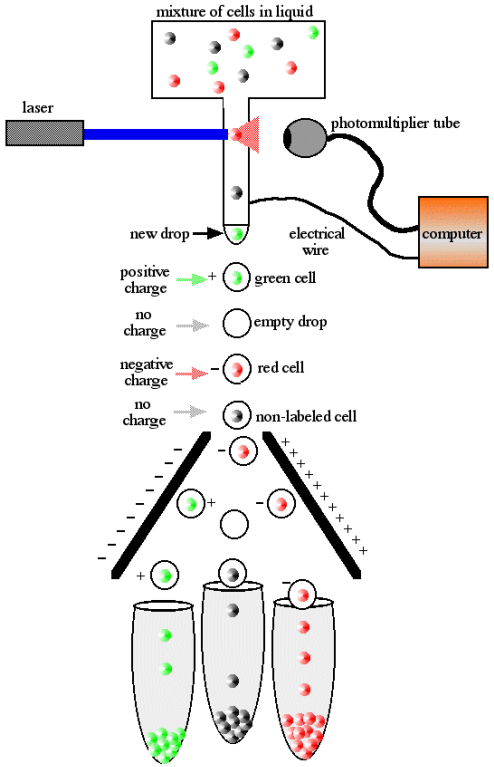
Methods

FACS analysis



CD4+CD28null T cells in PBMCs, SFMCs and ST-derived cells (CD4, CD28, CD3)

<u>phenotypical characterization:</u> CD25, CD45RO, CD62L, TCRαβ, CD11a, CD27, CD44, CD45RA, CD49d

<u>intracellular staining:</u> granzyme A & B, perforin

http://www.bio.davidson.edu/genomics/method/FACS.html

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Methods

Isolation of CFSE labelling of sorted T cells

$CD4^+CD28^{null}$, $CD4^+CD28^+$, $CD4^+CD25^{high}$ T cell populations were sorted

labelling of T cell subsets with CFSE

CFSE-based proliferation assay

CFSE-labelled $CD4^+CD28^{null}$ T cells + autologous PBMCs (loaded with tetanus toxoid, myelin basic protein, human collagen type II, CMV) + IL-2

evaluation of proliferation rate by FACS analysis

negative control: unloaded PBMCs
positive control: anti-CD3 mAB

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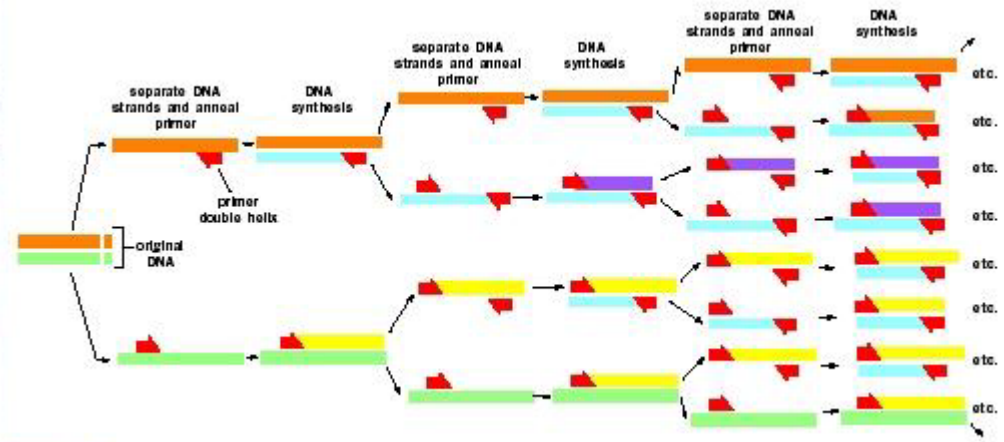
Methods

Analysis of TCR BV gene usage and CD3R fragment length of CMV-reactive $CD4^{+}CD28^{null}$ T cells

semiquantitative TCR BV analysis on CMV or anti-CD3 mAb-stimulated $CD4^{+}CD28^{null}$ T cells via PCR

CDR3 fragment length analysis via PCR



http://www.biologyreference.com/Ph-Po/Polymerase-Chain-Reaction.html

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Methods

Suppression assay

coculture of CFSE-labelled $CD4^+CD28^{null}$/$CD4^+CD28^+$ T cells + irradiated autologous PBMCs (+ $CD4^+CD25^{high}$ Treg cells)

stimulation with anti-CD3 mAb and IL-2

evaluation of proliferation via FACS analysis

ELISA

evaluation of INF-γ and GrB in cell culture supernatants

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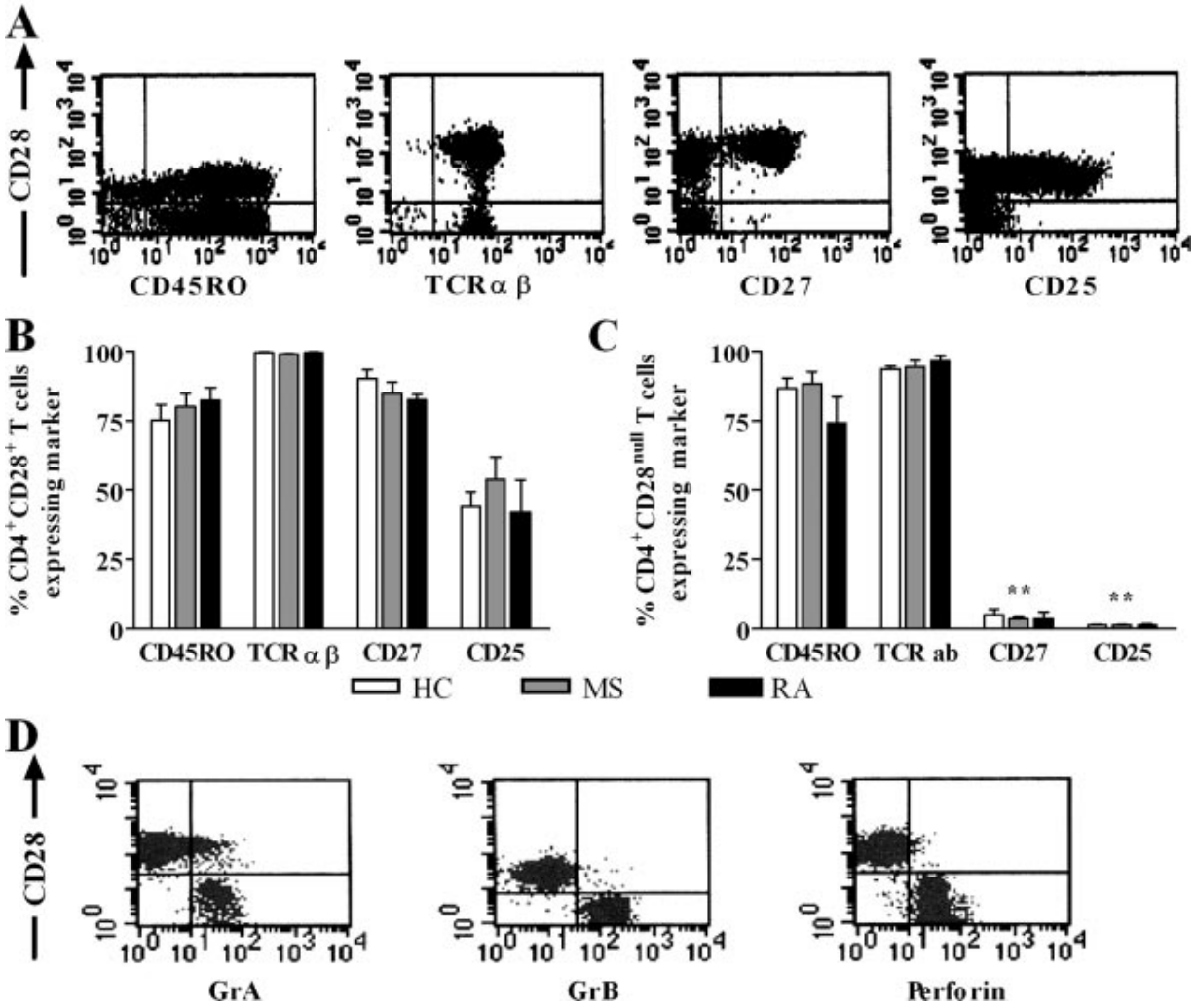
Results



→ *$CD4^{+}CD28^{null}$ cells express CD45RO & TCRαß equally to their $CD28^{+}$ counterparts, however, have lost expression of CD27 & CD25*

→ *$CD4^{+}CD28^{null}$ cells express the cytotoxic markers granzyme A, granzyme B & perforin*

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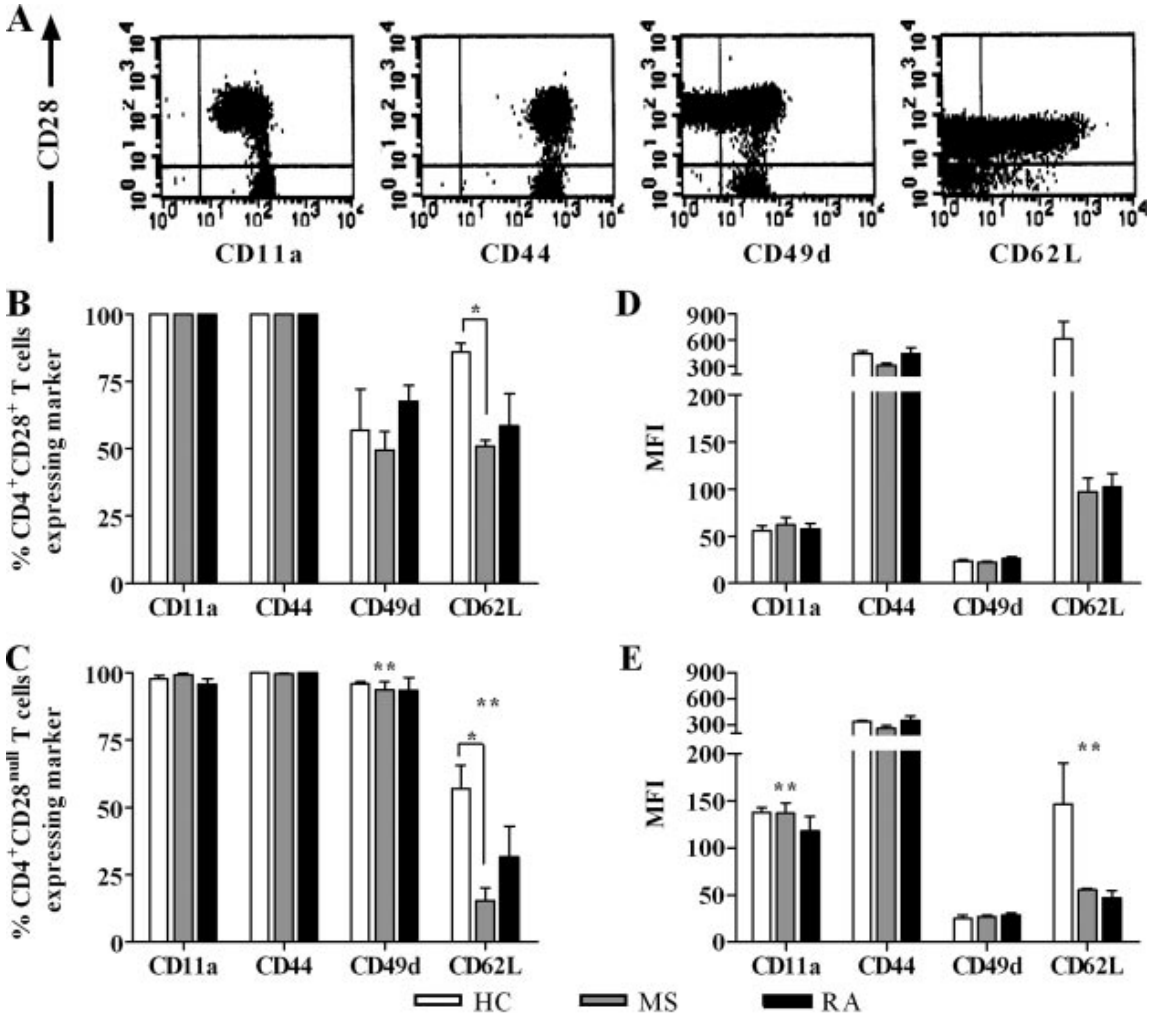
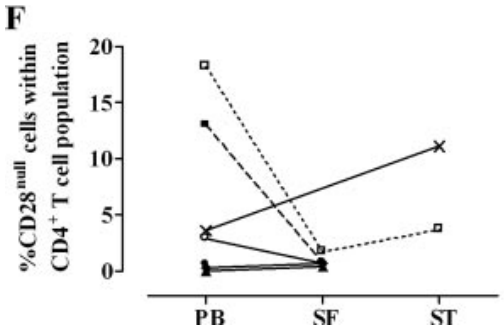
Results



→ *CD4⁺CD28^{null} cells express CD11a & CD44 equally to their CD28⁺ counterparts.*

→ *CD4⁺CD28^{null} cells express CD11a higher than CD28⁺ cells.*

→ *All CD4⁺CD28^{null} cells express CD49d, however, more CD4⁺CD28⁺ cells express CD62L.*



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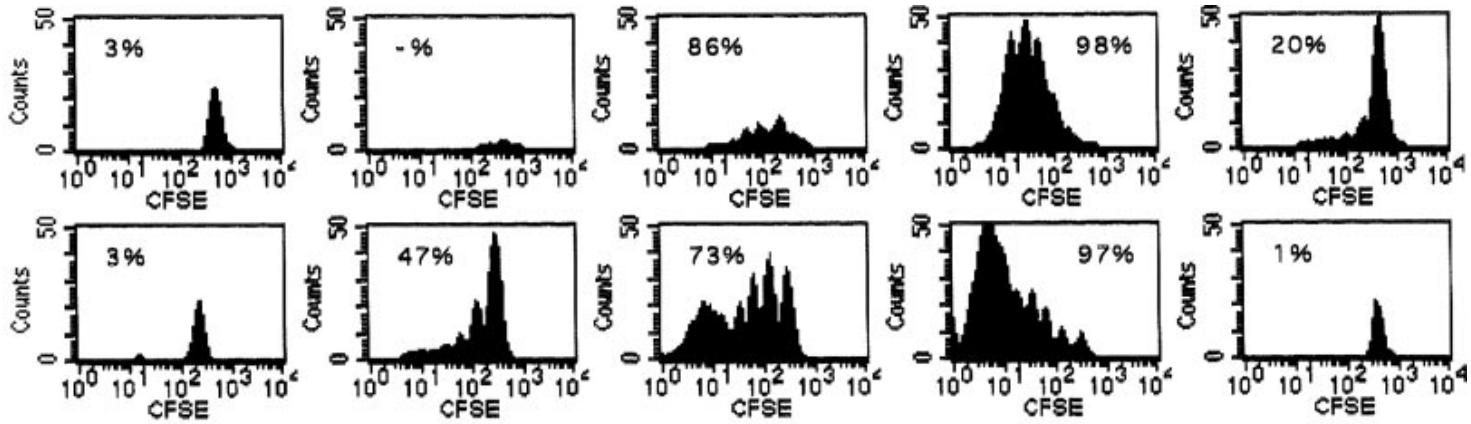

Feeders	+	+	+	+	-
Anti-CD3 (2 µg/ml)	-	+	+	+	+
IL-2 (U/ml)	**0**	**0**	**0.1**	**2**	**2**

→ *Addition of IL-2 is essential for survival & proliferation of CD4⁺CD28^{null} cells.*

→ *CD4⁺CD28^{null} cells can proliferate in the absence of costimulation.*

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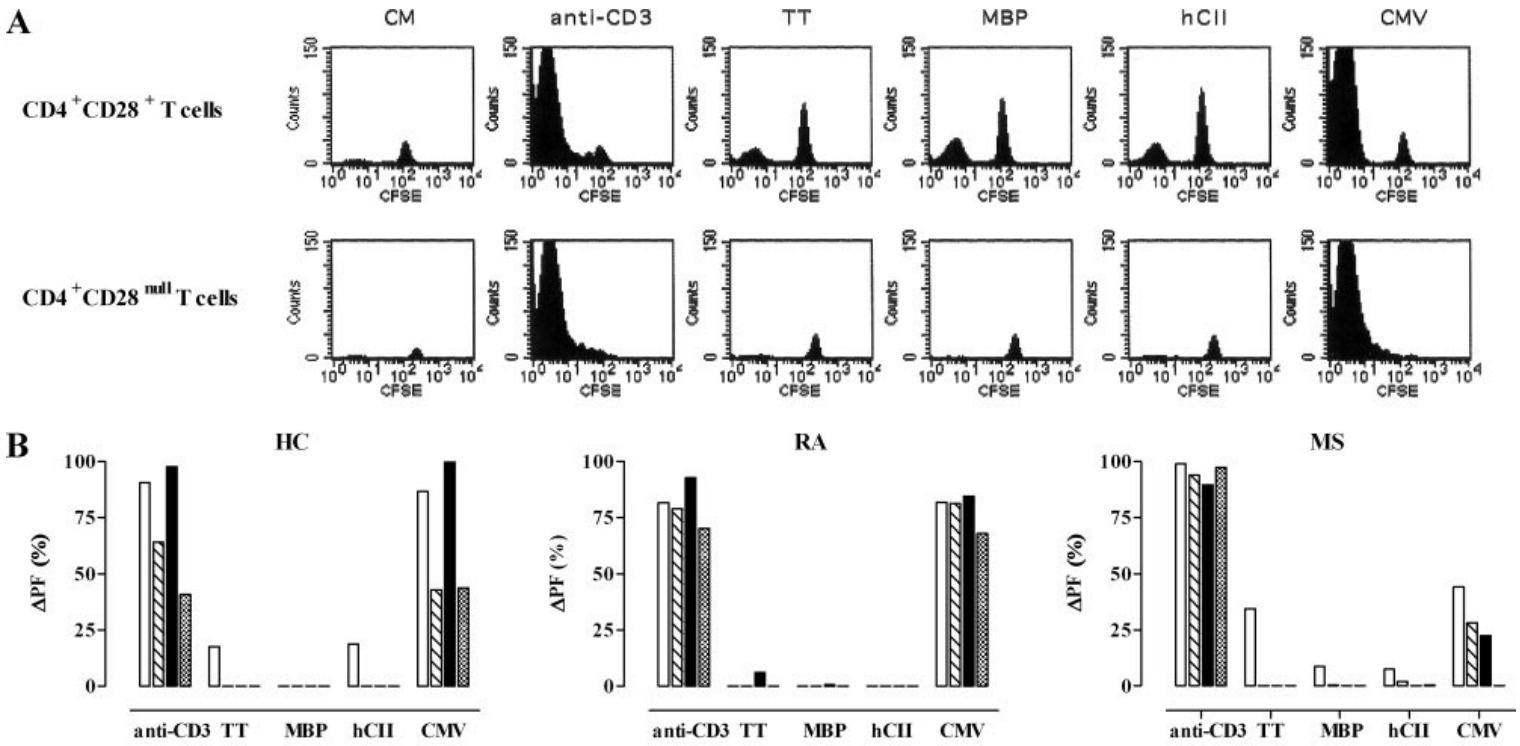

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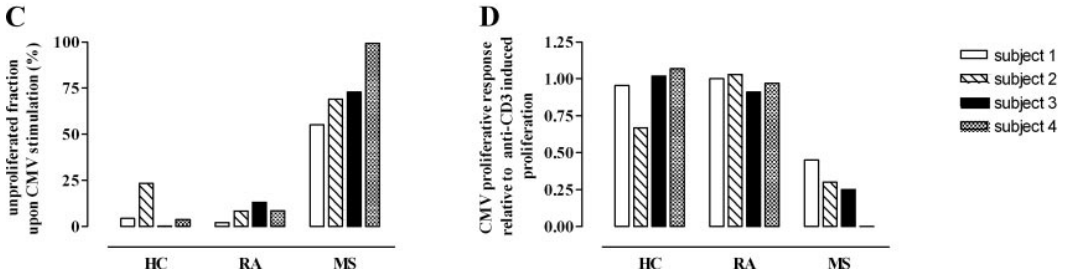

→ *MS patients display a high fraction of CMV-unresponsive cells.*

→ *In MS patients the level of CMV response relative to anti-CD3 induced proliferation was reduced.*

	anti-CD3		TT		MBP		hCII		CMV	
	IFN-γ	GrB	IFN-γ	GrB	IFN-γ	GrB	IFN-γ	GrB	IFN-γ	GrB
HC1	++	−	−	−	−	−	+/−	++	+++	+++
HC2	++	−	−	−	−	−	−	−	++	+++
HC3	++	+++	+	+	+/−	+/−	−	+/−	+++	+++
HC4	++	+++	−	−	−	−	+/−	−	+++	+++
RA1	++	++	−	−	−	+/−	−	+	++	+++
RA2	+	+++	−	−	−	−	−	+/−	++	+++
RA3	+	+/−	+/−	+	−	−	−	−	++	++
RA4	+	−	+/−	−	+/−	−	+/−	+/−	+	++
MS1	+++	+++	+	+	−	−	−	−	+++	+++
MS2	+++	+++	−	−	−	−	+/−	−	++	++
MS3	++	++	−	−	−	−	−	−	+	+
MS4	++	+++	−	−	−	−	−	−	−	−

[a] IFN-γ and GrB secretion were measured by means of ELISA in the culture supernatants of $CD4^+CD28^{null}$ T cells stimulated with various Ags. The amount of secreted IFN-γ or GrB was divided arbitrarily into five levels: −, <25 pg/ml; +/−, 25–100 pg/ml; +, 100–500 pg/ml; ++, 500–1000 pg/ml; and +++, >1000 pg/ml.

→ *Large amounts of INF-γ & GrB can be detected in supernatant upon CMV stimulation*

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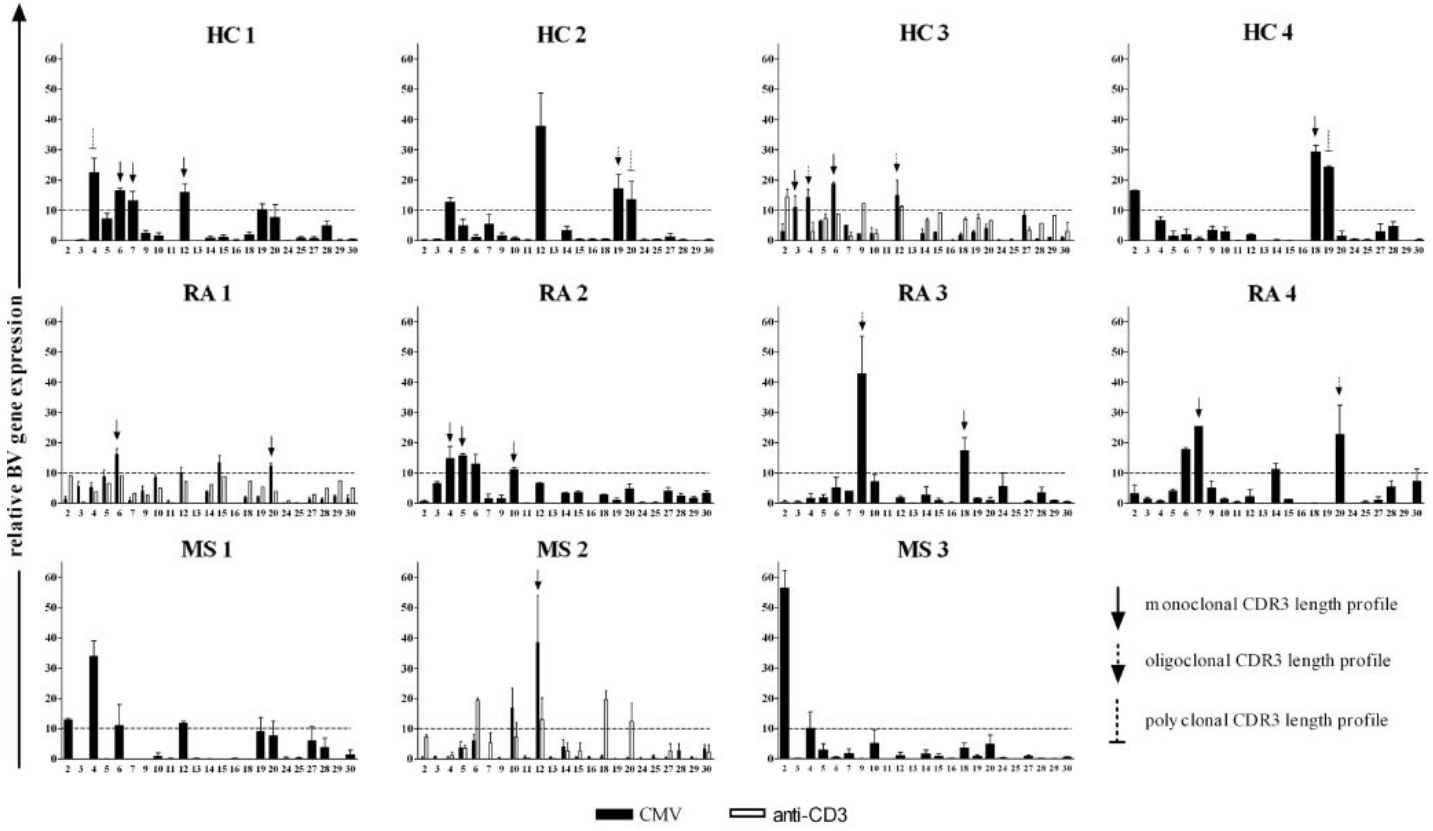

→ In general, a wide variety of TCR BV genes was expressed, however, in some individuals one single predominant TCR BV family could be identified. This may be associated with a diminished CMV-induced immune response.

→ Anti-CD3 stimulation resulted in a similar repertoire of TCR BV genes.
→ In MS patients some TCR BV genes were expressed differently, suggesting a fraction of not CMV specific $CD4^{+}CD28^{null}$ cells.

→ Overexpressed TCR BV genes upon CMV-stimulation displayed a monoclonal or oligoclonal CDR 3 length profile, suggesting that $CD4^{+}CD28^{null}$ cells are clonally expanded cells

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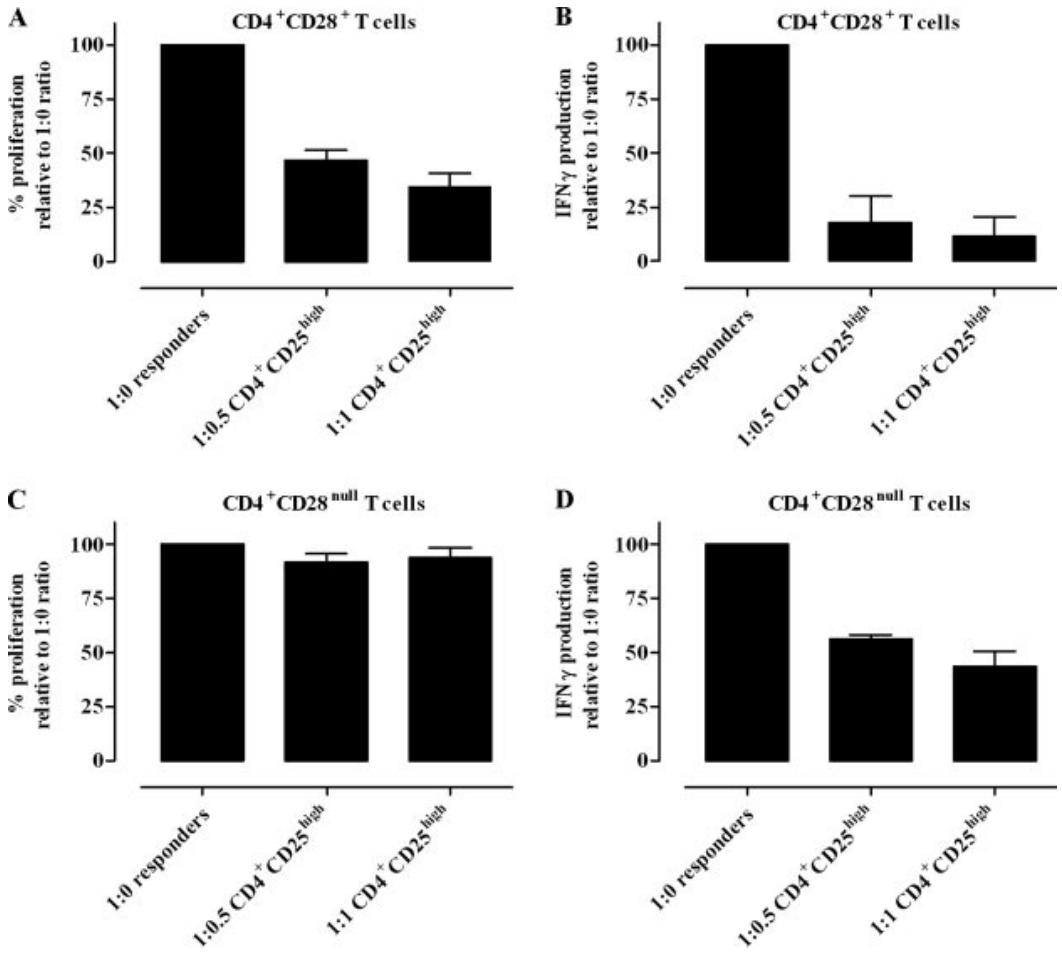

→ *Proliferation of $CD4^{+}CD28^{null}$ cells cannot be inhibited by $CD4^{+}CD25^{high}$ Tregs.*

→ *However, INF-γ production can be inhibited by $CD4^{+}CD25^{high}$ Tregs.*

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Conclusion

$CD4^+CD28^{null}$ cells display several features of pathogenic cells and are less susceptible to regulation by Tregs.

However, no reactivity to candidate autoantigens could be demonstrated.

CMV is the driving force behind the differentiation of $CD4^+$ cells in RA patients and HC.

$CD4^+CD28^{null}$ cells posses the capacity to infiltrate into tissues.

$CD4^+CD28^{null}$ cells of RA and MS patients do not differ from those in HC.