Children with Down syndrome show quantitative, phenotypical and functional differences of effector T-cells compared to immunocompetent controls

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Introduction and hypothesis

Trisomy 21 is not only associated with an increased susceptibility to respiratory infections suggesting a deficiency of the adaptive immune system but is also associated with an higher incidence of autoimmune disorders.

This study focuses on the investigation of the antigen-specific humoral and cellular immune response as well as the phenotypical cell features and quantitative analysis of lymphocyte subpopulations in children with Down syndrome (DS) compared to age-matched immunocompetent controls (C).

Subjects and methods

- **Subjects** (**Table 1**)
  - 40 children with Down syndrome (age: 7.38 ± 1.11 years)
  - 51 controls (age: 8.80 ± 0.97 years)
- **Methods**

1. Antigen-specific stimulation of T-cells (**Fig. 1**)
   - Functional and phenotypical characterization

   Whole blood samples were stimulated for 6h with specific antigens of cytomegalovirus (CMV), varizella zoster virus (VZV), mycobacteria (PPD) and polyclonal stimulated with Staphylococcus aureus Enterotoxin B (SEB). After 2h of incubation, brefeldin A was added to inhibit secretion of induced cytokines.
2. Cell-surface staining of lymphocyte subpopulations
   - Quantitative analysis
3. Quantification of humoral immune response using ELISA (CMV and VZV IgG)

Results

- Children with DS showed a higher proportion of NK-cells within the lymphocytes whereas the B-cell percentage was decreased and the percentage of T-cells did not differ in both groups (**Fig. 2**).
- Within the T-cell population, children with DS revealed less CD4+ and more CD4+CD8+ double positive T-cells (**Fig. 3**).
- Within the CD4+ T-cells, a higher proportion of regulatory T-cells, TH17-cells, and a higher TH1/TH2 ratio was detectable in children with DS (**Fig. 4 A,B**).
- Children with DS showed a higher expression of the anergy markers PD-1 and CTLA-4 on CD4+T-cells (**Fig. 4C**).
- Both groups showed an age-appropriate extent of endemic infection with CMV, VZV and mycobacteria and no pronounced differences in antigen-specific immune response were detectable (**Table 2, Fig.5**).
- Frequency of polyclonally activated effector T-cells in children with DS were significantly higher than in controls (**Fig.5**).

Conclusion

- Besides a general proportional shift of leukocyte and lymphocyte subpopulations, effector T-cells seem to be functionally impaired which may contribute to a higher susceptibility towards infections.
- The simultaneously higher fraction of reactive effector T-cells may represent a compensatory effect of functional anergy and/or be a consequence of a more pronounced history of infections.

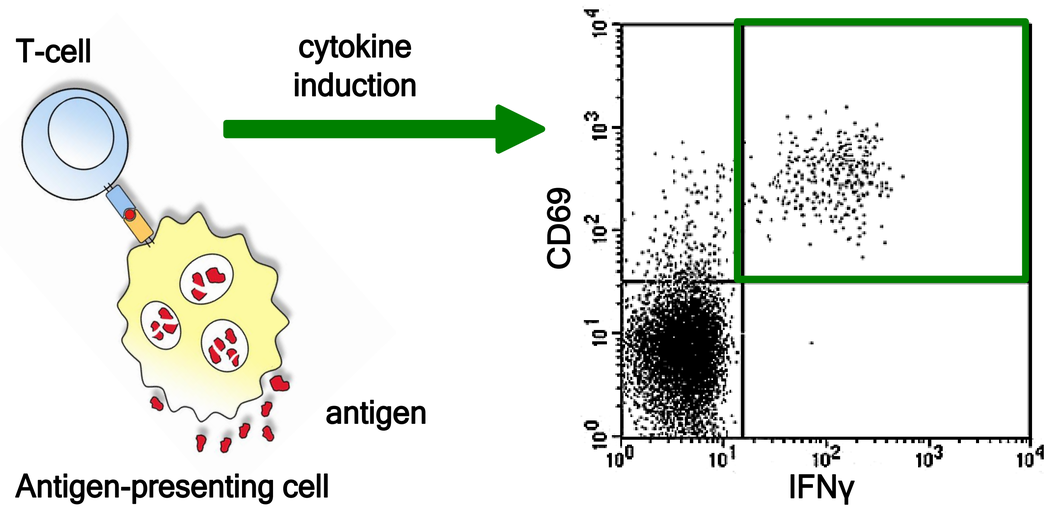

Fig. 1: Antigen specific stimultion of T-cells. T-cells of heparinized whole blood were stimulated in vitro with antigens from CMV, VZV and PPD. Stimulations with uninfected control lysate and SEB served as negative and positive controls, respectively. Reactive T-cells were analyzed based on surface expression of the activation marker CD69 and the induction of interferon-gamma (IFNγ) .

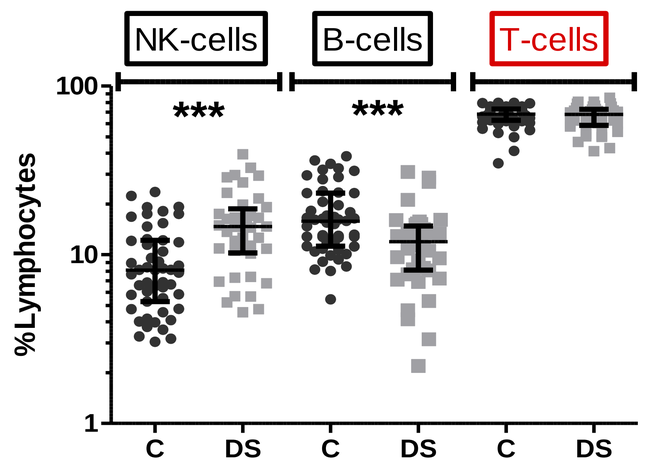

Fig. 2: Altered distribution of lymphocyte subpopulations in children with DS (n=40) compared to controls (C, n=51): NK-cells (CD56+CD16+; p=0.0002), B-cells (CD19+; p=0.0007) und T-cells (CD3+; ns).

Table 1: Characteristics of study population

Characteristics	HC	DS
Number	51	40
Age	8.798 ± 0.9703	7.387 ± 1.109
sex (m/f)	25/26	23/17
Respiratory tract infections	8	8
Heart defects	11	25
Heart operation	5	17
Autoimmune disorders*	4	11

* Autoimmune disorders: Autoimmune thyreoditis (Hashimoto, Graves disease), psoriasis, autoimmune thrombozytopenia, coeliac disease.

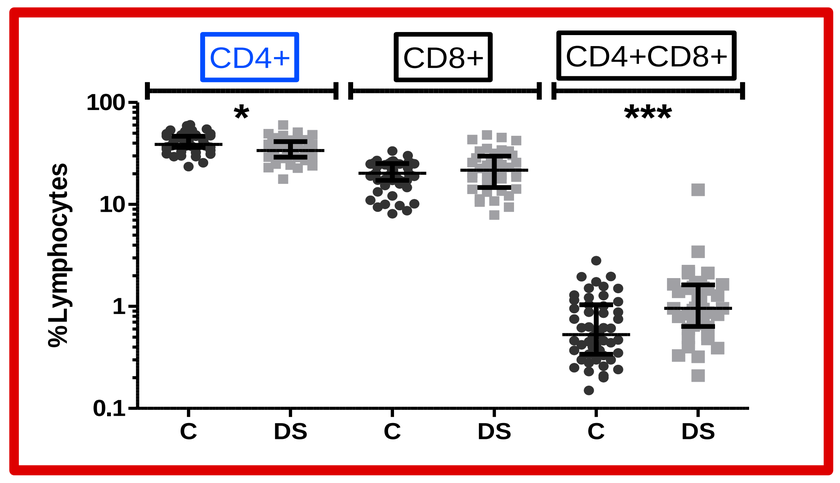

Fig.3: Quantitative analysis of T-cell subpopulations in children with DS (n=40) compared to controls (C, n=51). Within the T-cell subpopulation, children with DS revealed a higher percetage of CD4+CD8+ T-cells (p=0.0008) and a lower percentage of CD4+ single positive T-cells (p=0.0122). No difference in the proportion of CD8+ T-cells was detectable.

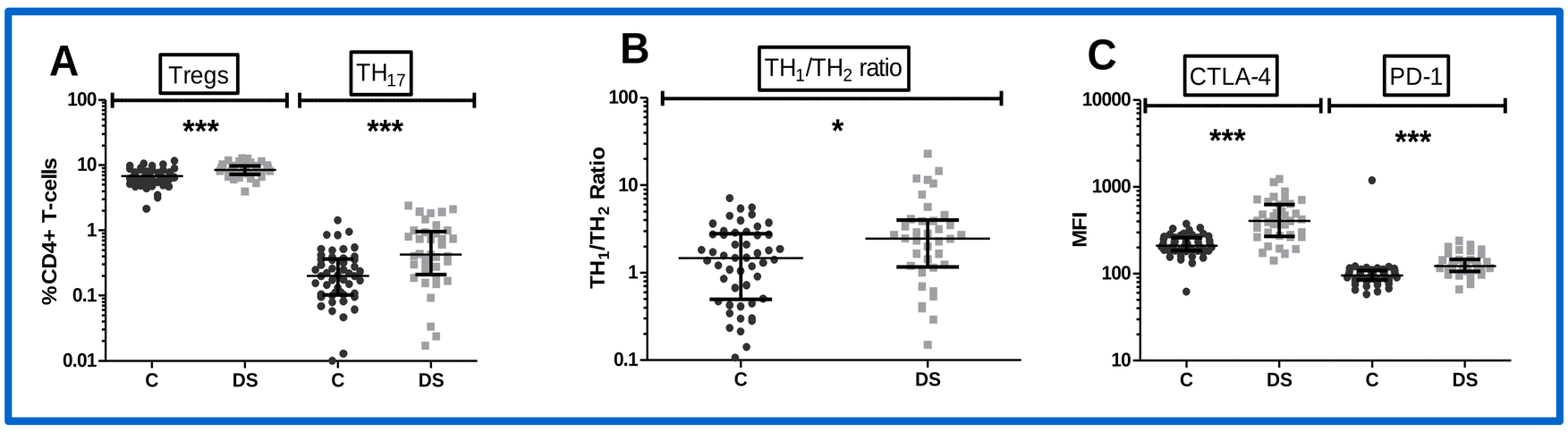

Fig. 4: Detailed analysis of CD4+T-cell population in children with DS and controls. A Children with DS showed a higher percentage of regulatory T-cells (Tregs: CD25+CD127low CD4+ T-cells; C (n=51) vs DS (n=40); p<0.0001) and TH_{17} -cells (IL-17+CD4+ T-cells after polyclonal stimulation; C: n=50 vs DS: n=37; p=0.0004) within the CD4+ T-cell population. **B** TH_1/TH_2 ratio was quantified by cytokine expression of IFNγ and IL-4 after polyclonal stimulation (C (n=50) vs DS (n=37); p=0.0253). **C** Higher expression levels (Median Flourescence Intensity, MFI) of the anergy markers CTLA-4 (p<0.0001) and PD-1 (p<0.0001) were detectable in children with DS.

Table 2: Correlation of humoral and cellular immune response

CMV controls		CMV IgG +	CMV IgG -
IFNγ+/CD69+ T-cells	positive >0.05%	7	0
	negative <0.05%	0	43

VZV controls		VZV IgG +	VZV IgG +/-	VZV IgG -
IFNγ+/CD69+ T-cells	positive >0.02%	22	3	1
	negative <0.02%	16	0	8

CMV Down syndrome		CMV IgG +	CMV IgG -
IFNγ+/CD69+ T-cells	positiv >0.05%	7	0
	negativ <0.05%	1	29

VZV Down syndrome		VZV IgG +	VZV IgG +/-	VZV IgG -
IFNγ+/CD69+ T-cells	positive >0.02%	20	4	1
	negative <0.02%	2	1	8

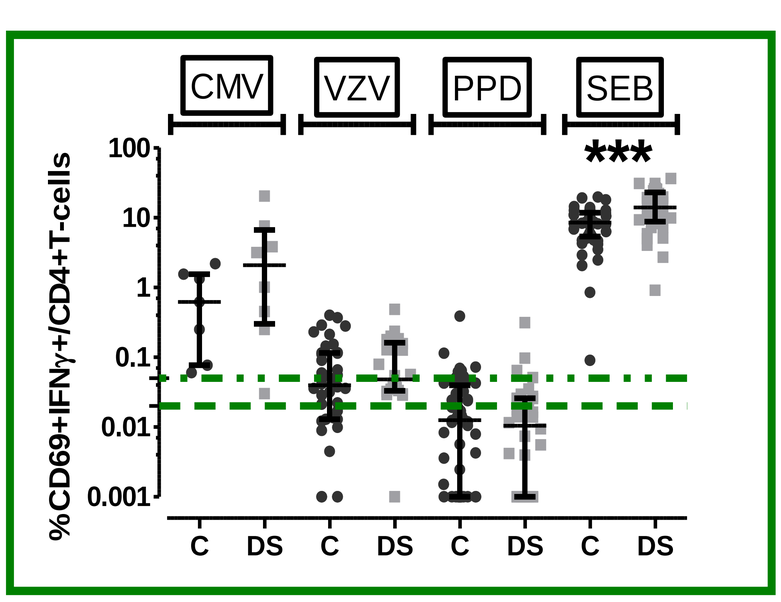

Fig. 5 Frequency of CD69+IFNγ+CD4+T-cells after antigen-specific and polyclonal stimulation (SEB) in children with DS and controls . Seropositive individuals after semiquantitative Elisa for CMV (C: n=7; DS: n=8) and VZV (C: n=38; DS: n=22) are shown. After antigen-specific stimulation, the percentage of reactive CD4+ T-cells did not differ in both groups, but children with DS showed a significantly higher frequency of reactive CD4+ T-cells after polyclonal stimulation with SEB.

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