ELISPOT: Implications for Molecular Mechanisms and Diagnosis

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Problems with Diagnosis of BeS or CBD

- CBD diagnosis requires BAL and biopsy
  - Invasive procedures
  - Imprecise since positive result requires presence of granulomatous inflammation **and** evidence of lymphocytosis (≥ 15%)
- Proliferation assay is not sensitive
- Proliferation assay does not differentiate between BeS and CBD stages

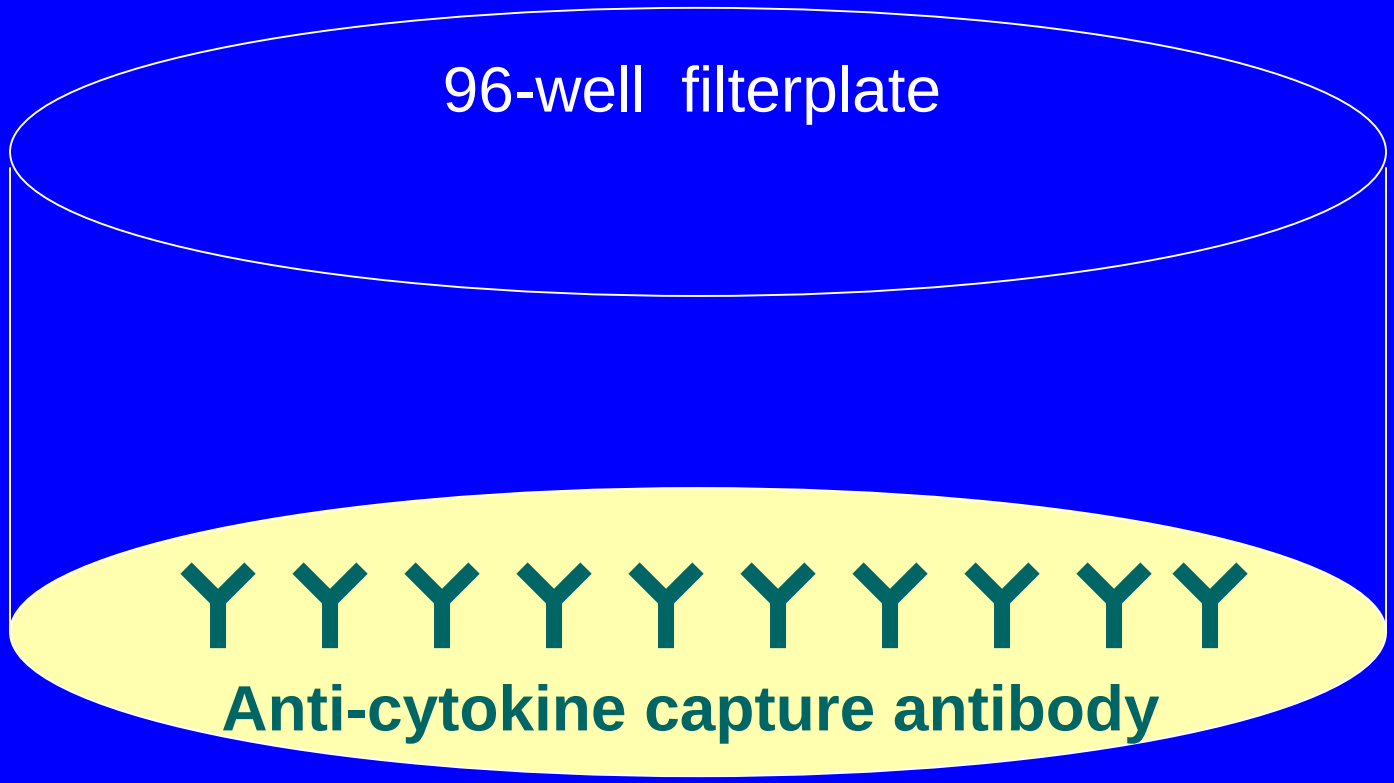
Potential Diagnostic Tests for Detection of Be Sensitization

- Assays that detect proliferation
  - BeLPT
  - Carboxyfluorescein diacetate-succinimidyl ester (CFSE)
  - BrdU and other flow cytometry-based assay
- Assays that detect cytokine secretion
  - ELISPOT
  - Intracellular cytokine detection

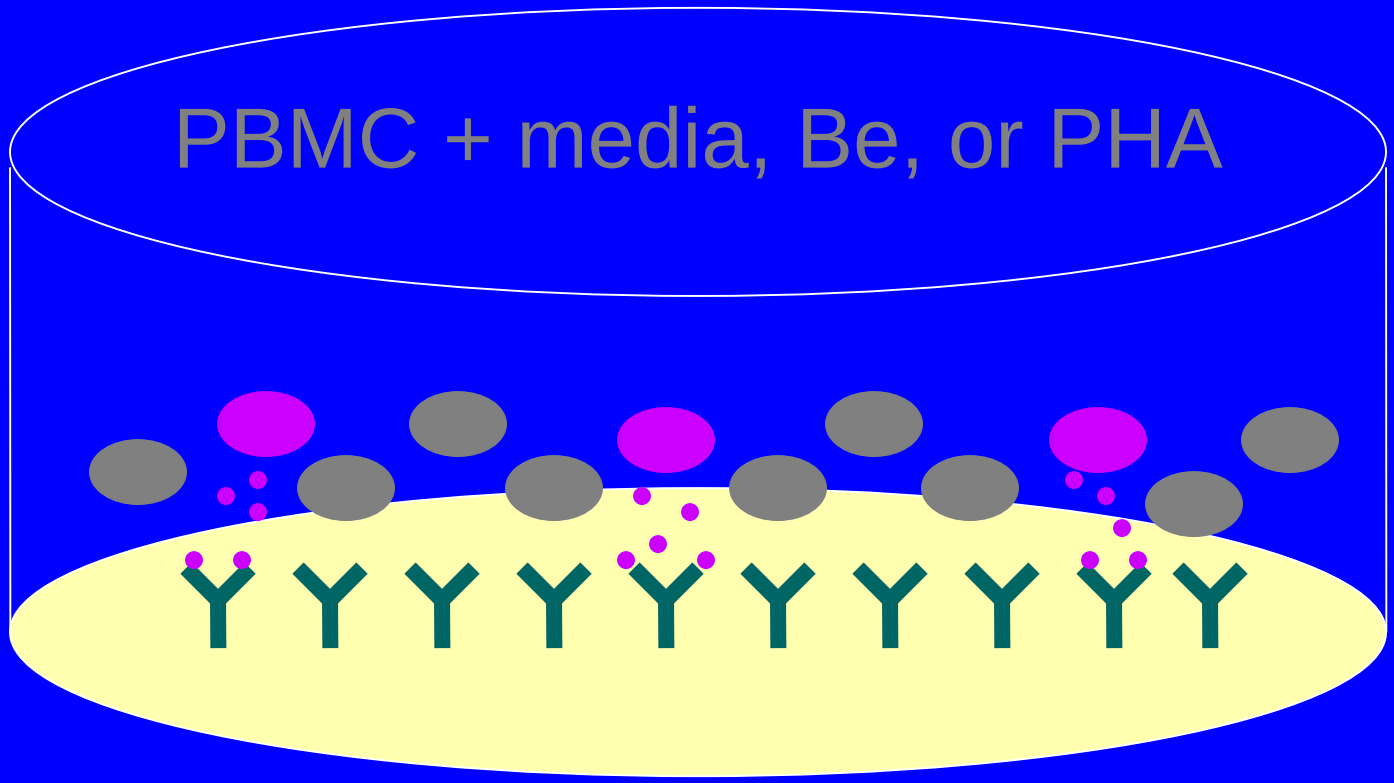
Questions

- Do CBD patients possess greater numbers of beryllium-specific $CD4^+$ T cells in blood compared to BeS subjects?
- Is progression from beryllium sensitization to CBD related to the absolute number of beryllium-specific $CD4^+$ T cells in blood?

ELISPOT Methods



ELISPOT Methods



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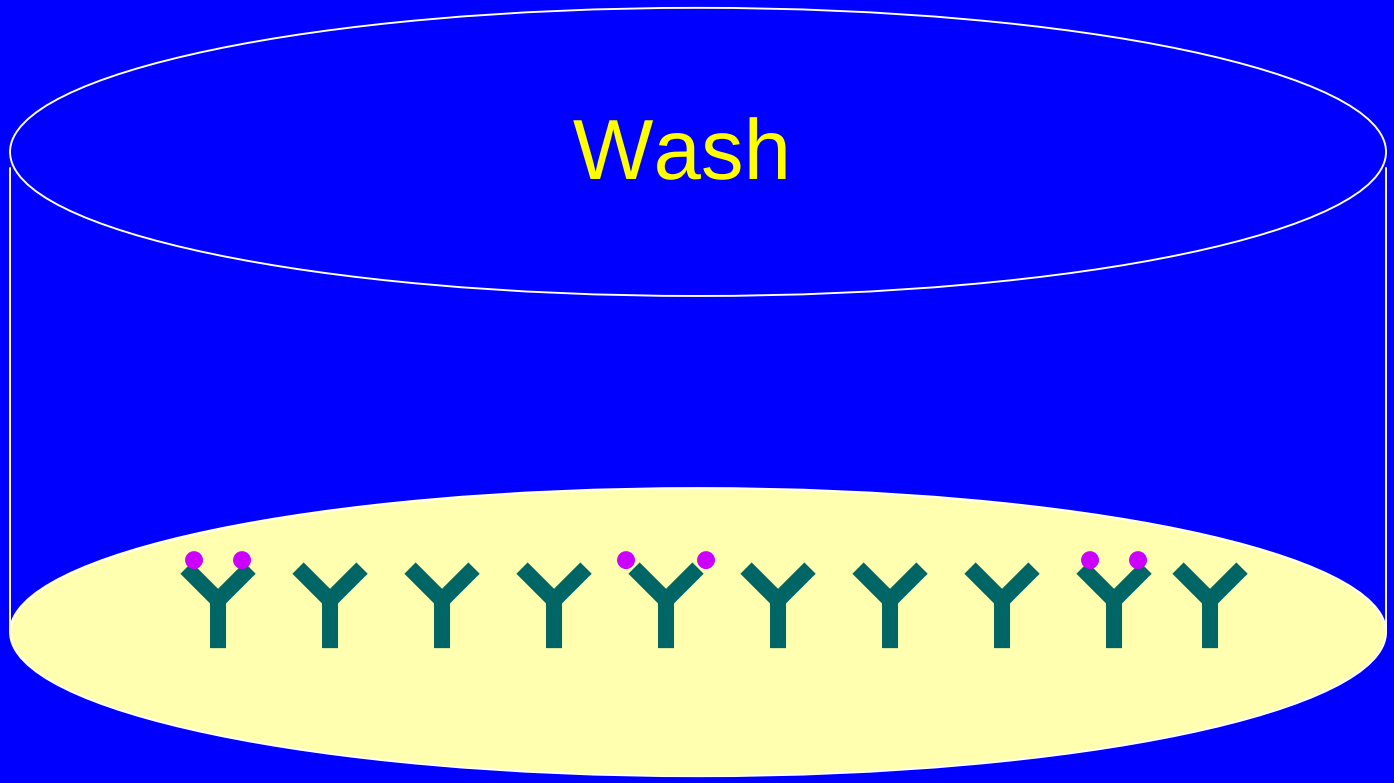

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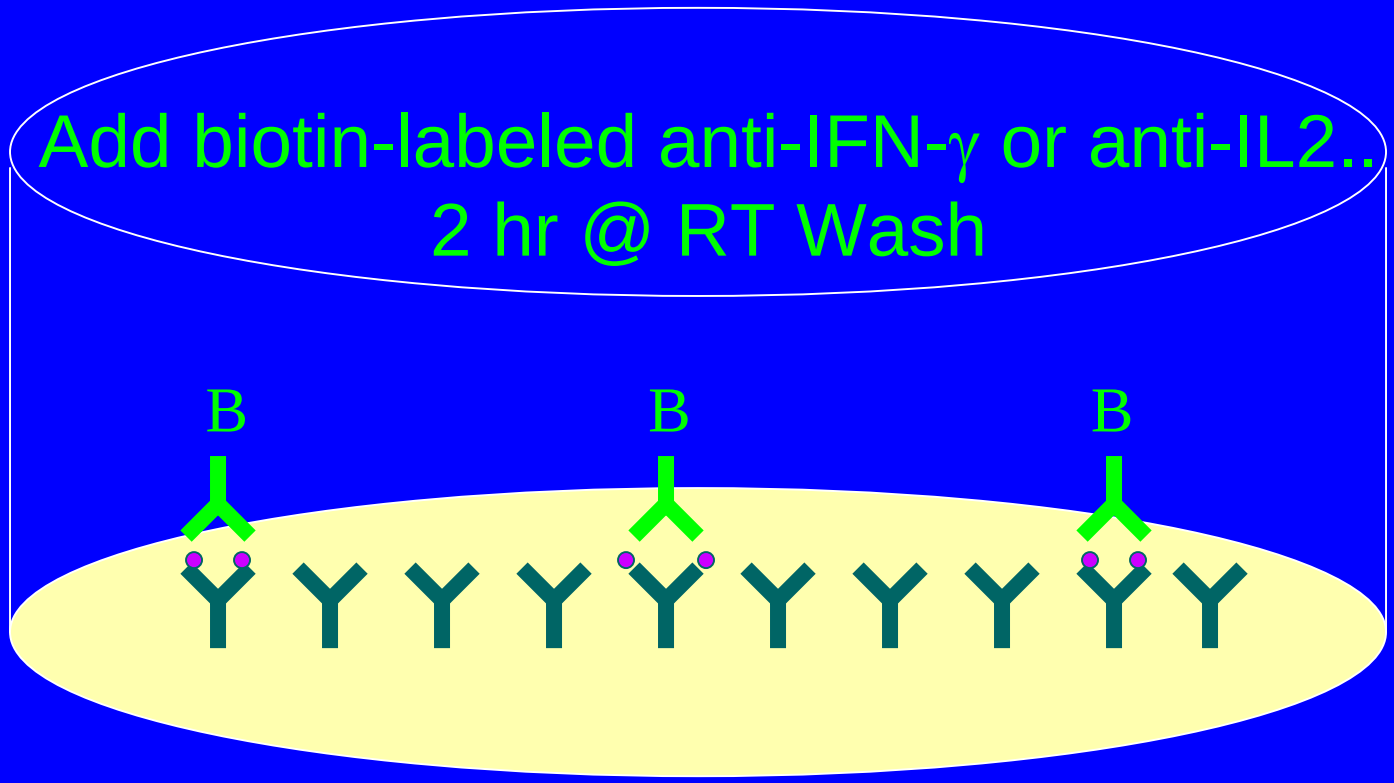

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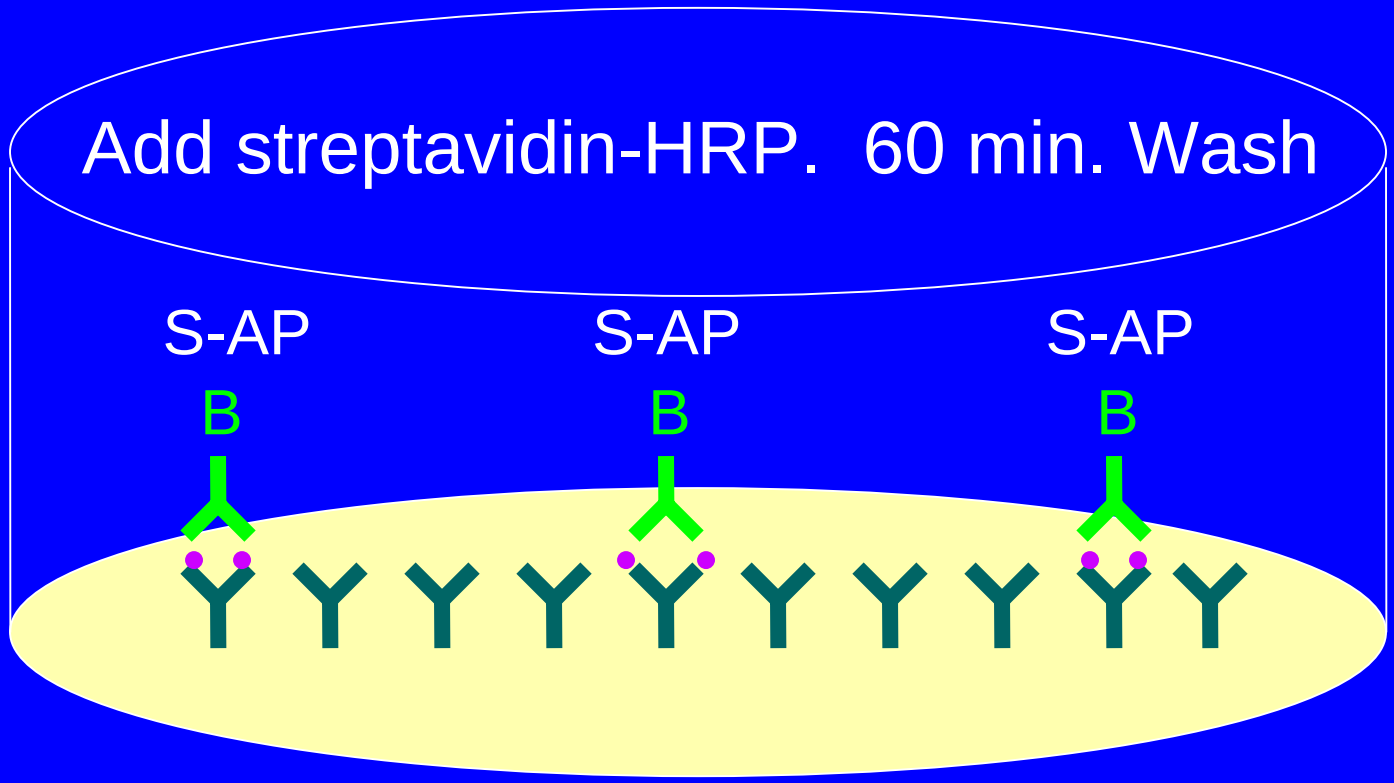

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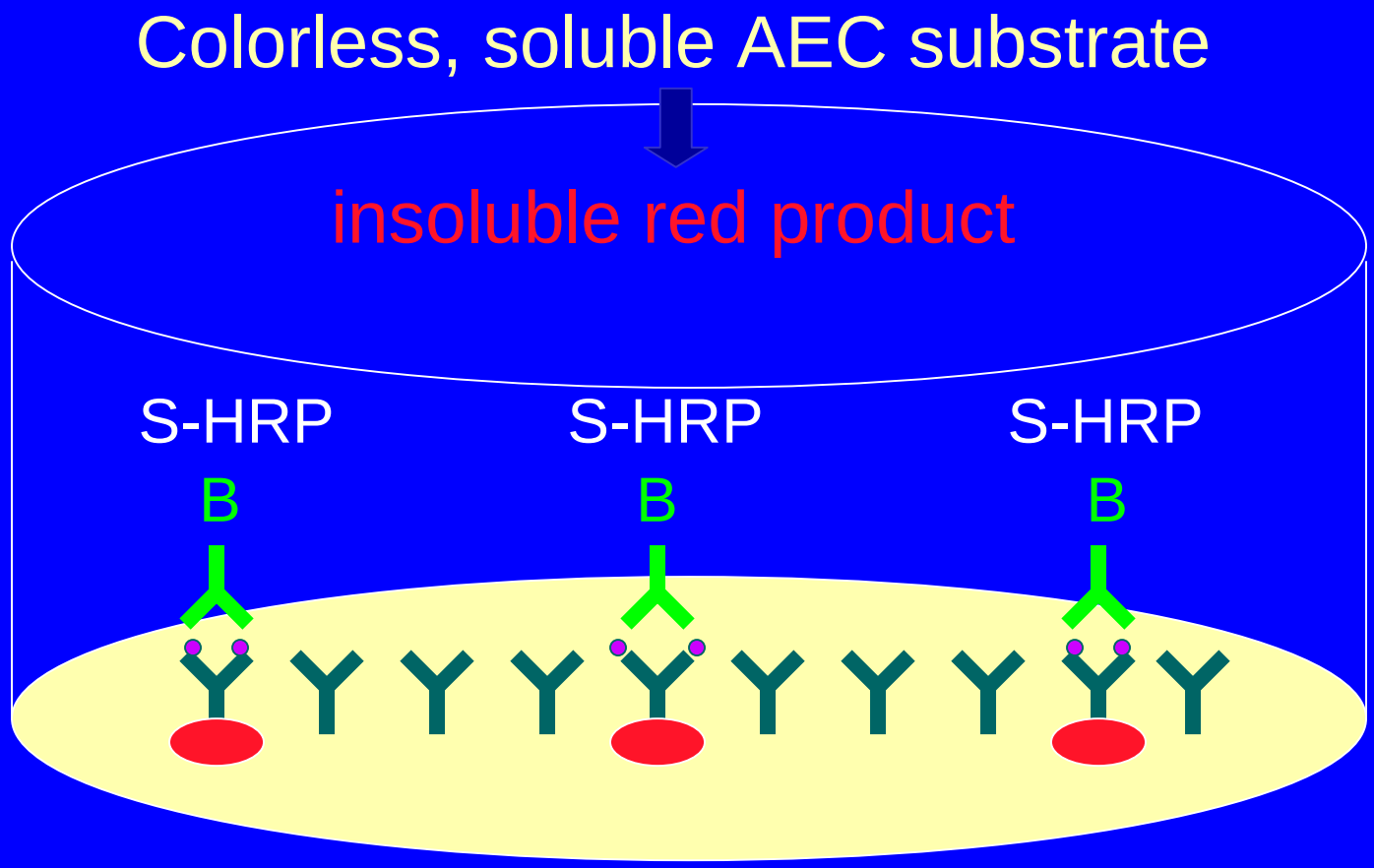

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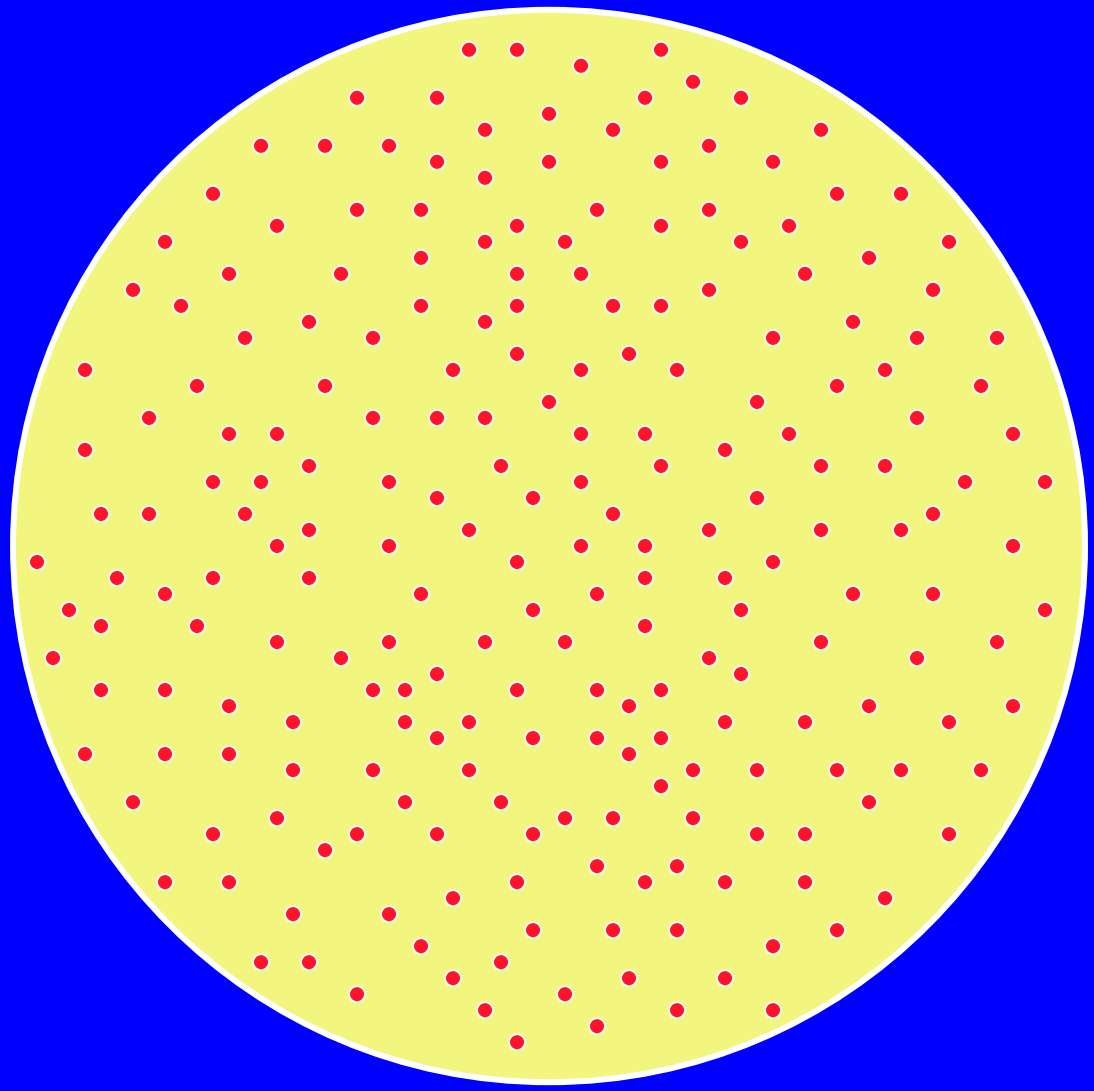
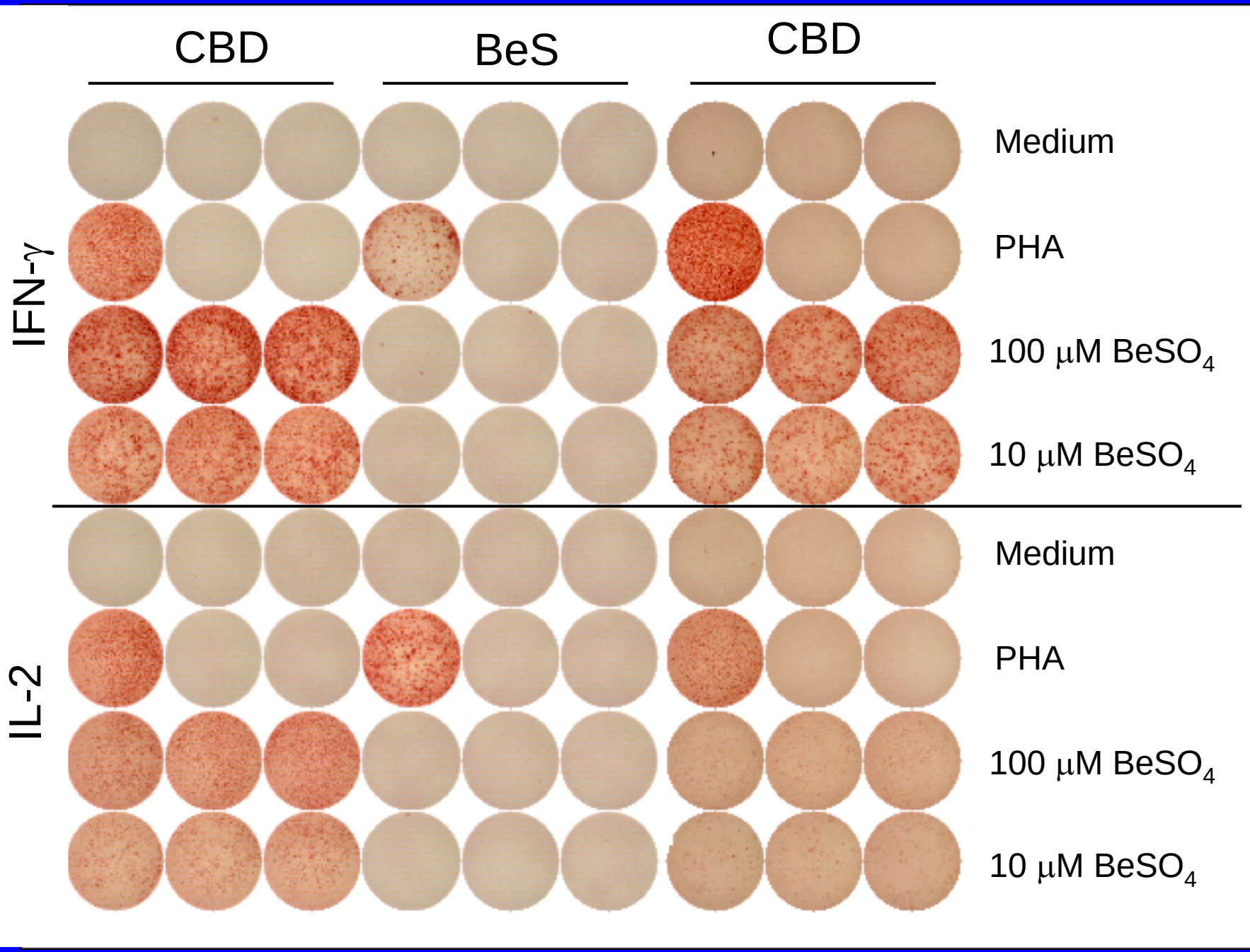
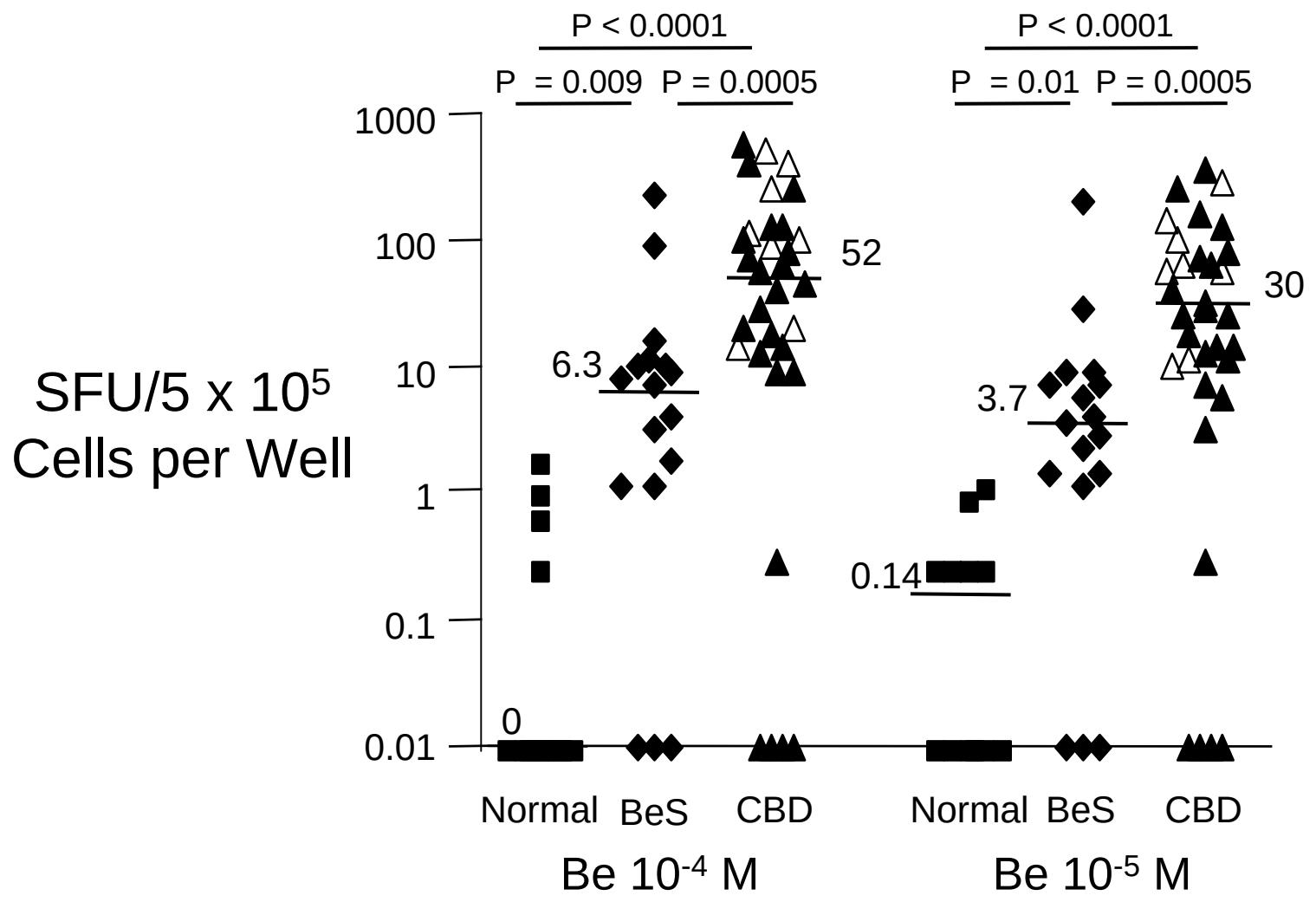



CBD
BeS
CBD
IFN-γ
IL-2
Medium
PHA
100 μM BeSO4
10 μM BeSO4
Medium
PHA
100 μM BeSO4
10 μM BeSO4

Demographics

Characteristics	BeS (n = 18)	CBD (n = 33)
Age (years)	57 (37 - 87)	62 (46 - 81)
Gender (M/F)	15/3	29/4
Race (C/AF/H)	14/2/2	25/2/6
Smoking Status (CS/FS/NS)	0/6/8	0/11/11
Years Since Diagnosis	2.6 (1.2 - 8.0)	7.1 (0 - 13.8)
Industry of Exposure (Nuclear/Ceramic/Other)	17/0/1	22/8/3
Treatment (Y/N)	0/18	8/25
BAL Cells		
WBC Count	18.5 (4.3 - 34.9)	**30.5 (6.5 - 230)**
Lymphocytes (%)	7.2 (1.7 - 21.9)	**21.5 (1 - 87.3)**

IFN-γ ELISPOT Assay



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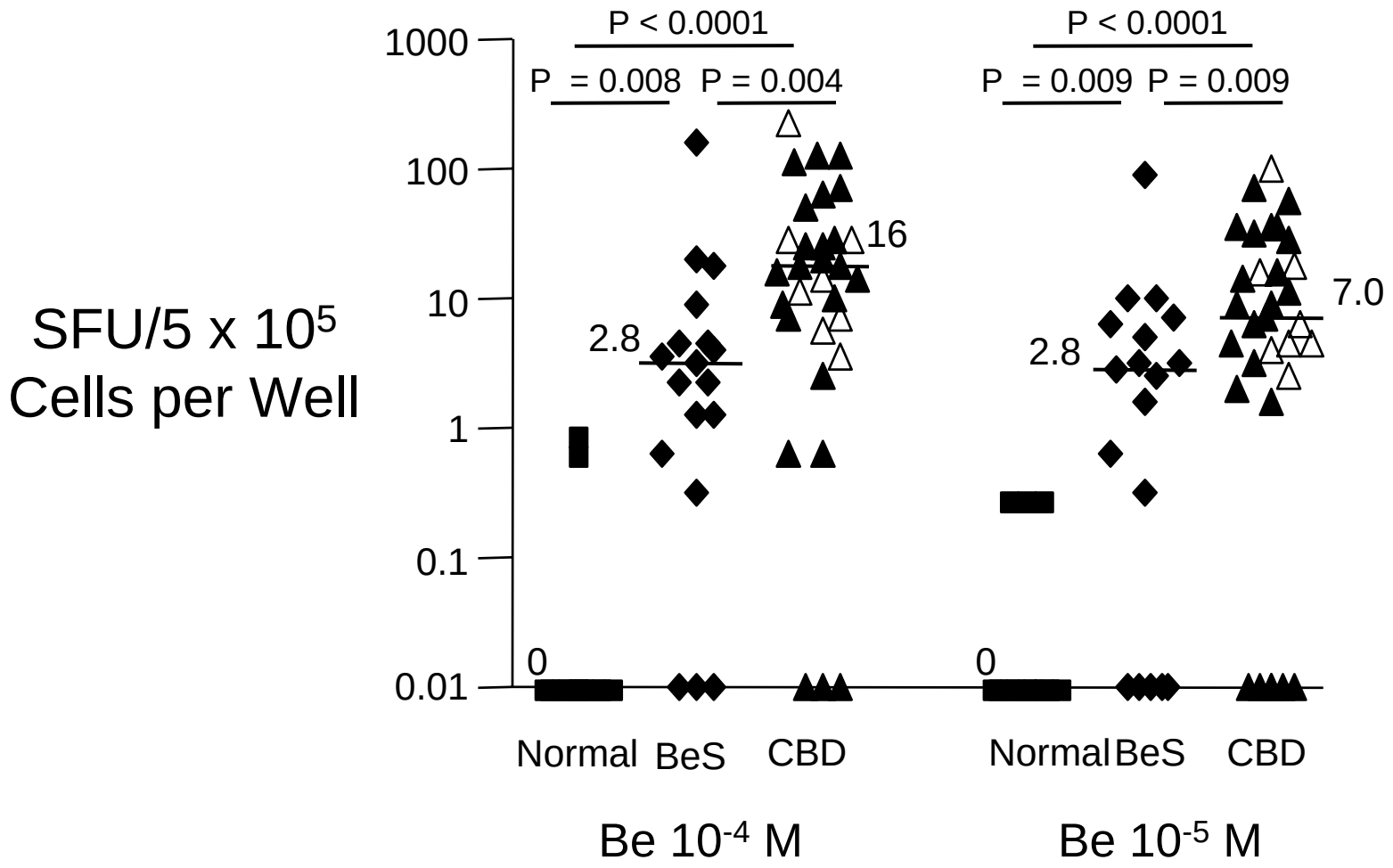
IL-2 ELISPOT Assay



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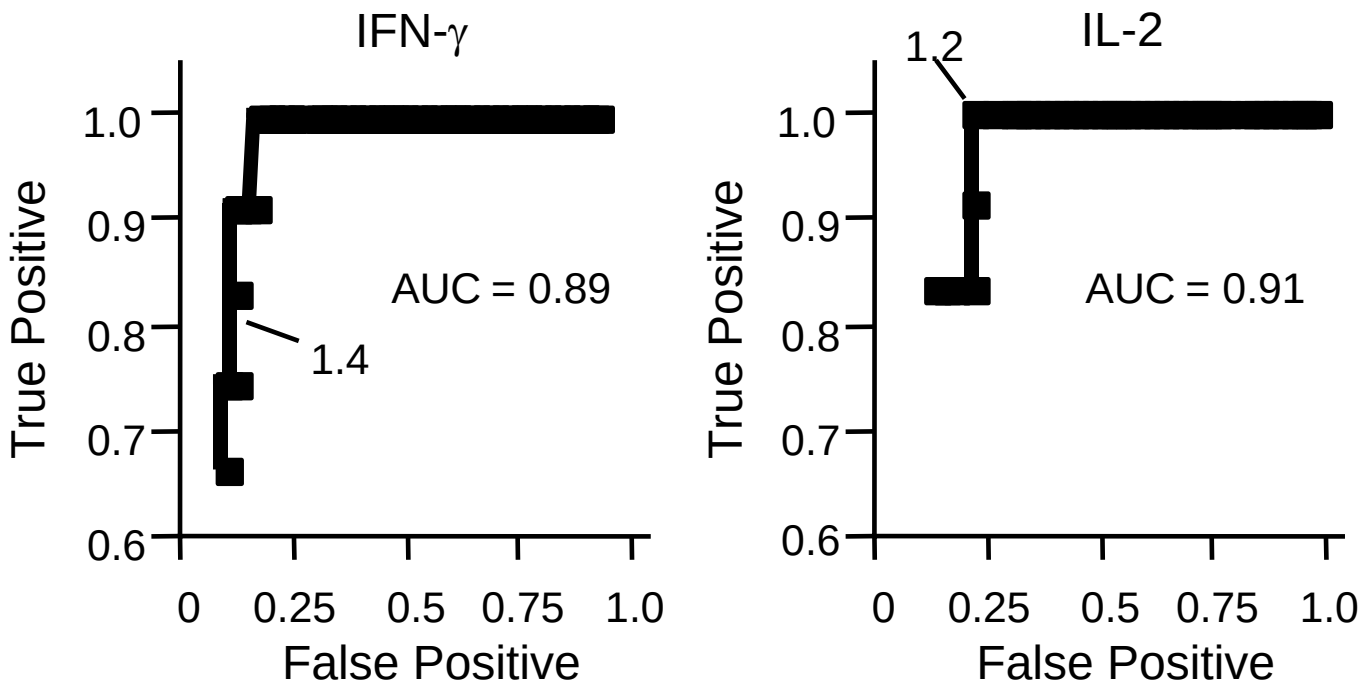
ROC Curves for IFN-γ and IL-2 ELISPOT



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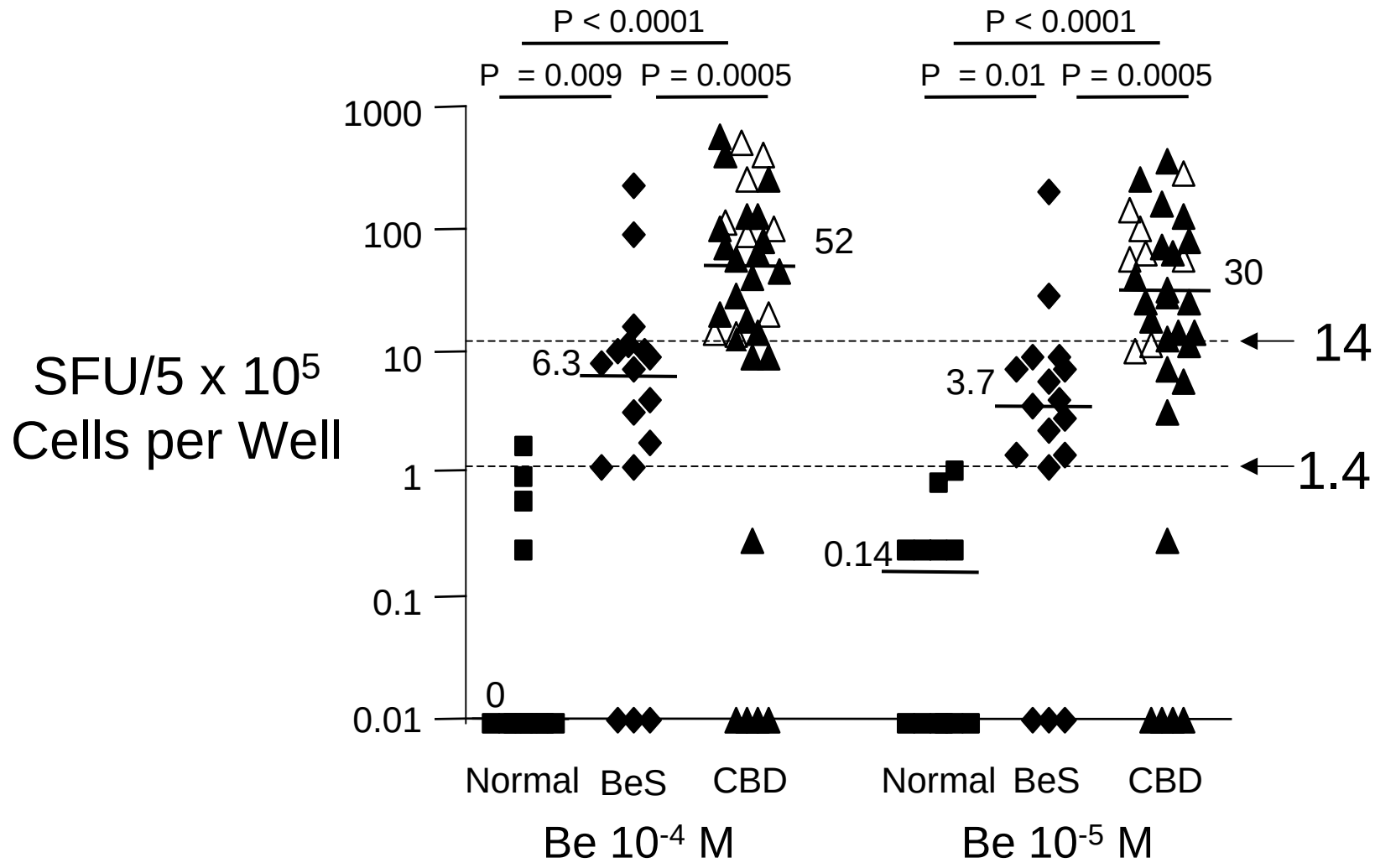
IFN-γ ELISPOT Assay



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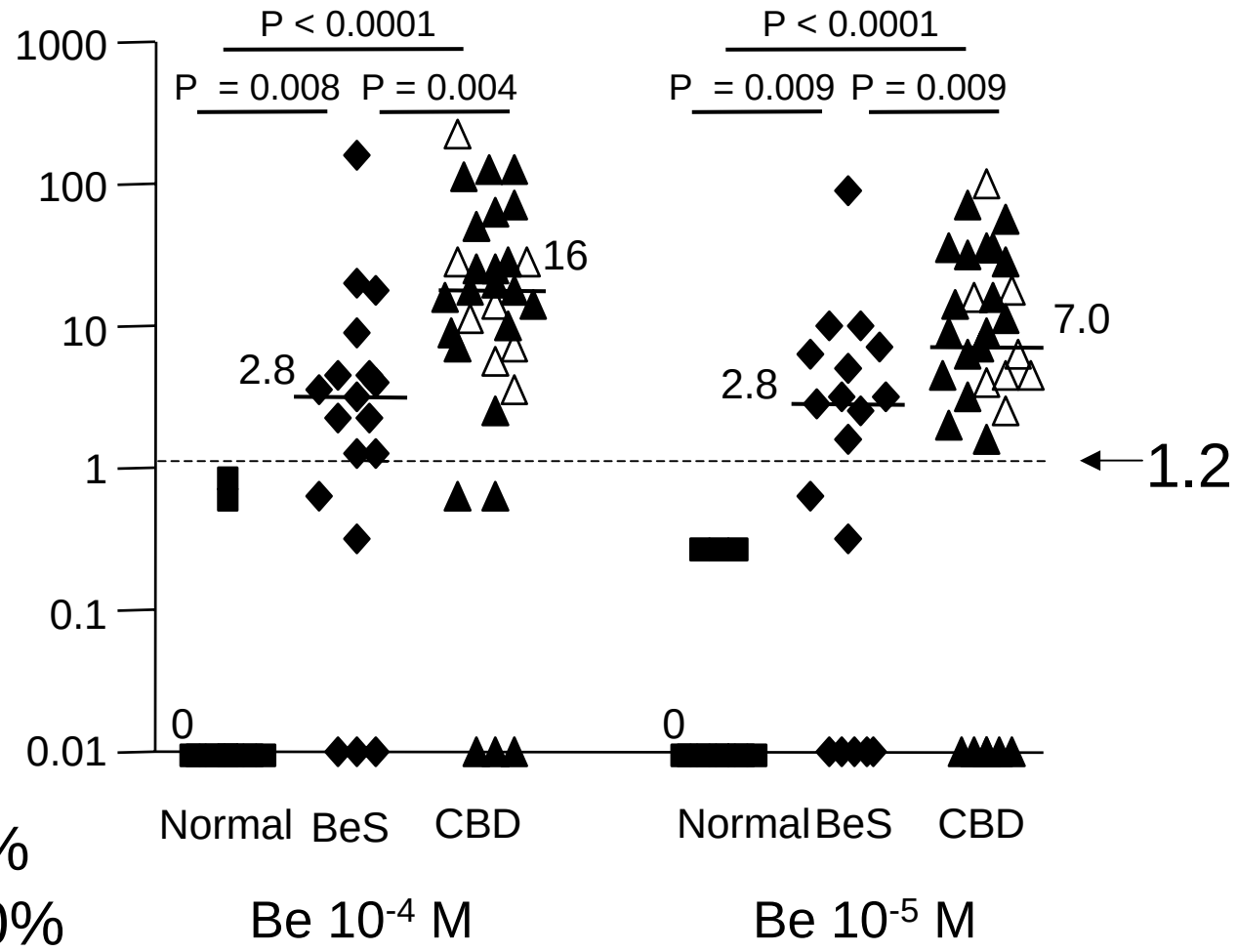
IFN-γ ELISPOT for Detection of Beryllium Sensitization

- Using a cut-point of 1.4 SFUs per 5 x 105 cells, IFN-γ ELISPOT has a sensitivity of 80% and specificity of 92%
- Using a cut-point of >14 SFUs per 5 x 105 cells for the differentiation of CBD from BeS, IFN-γ ELISPOT has a sensitivity of 78% and specificity of 88%

IL-2 ELISPOT Assay



SFU/5 x 10^5 Cells per Well

Sensitivity = 78%

Specificity = 100%

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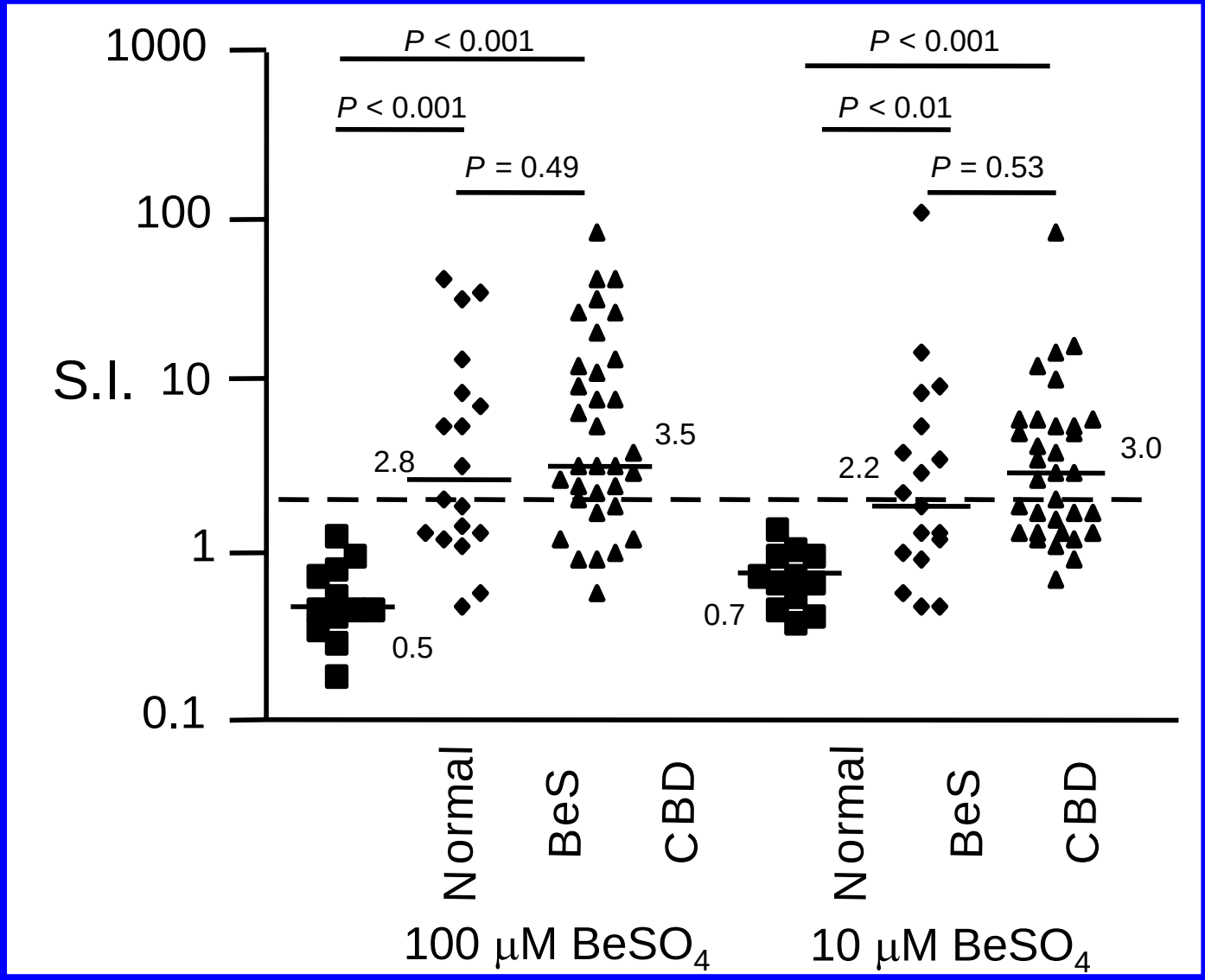
Lymphocyte Proliferation Assay



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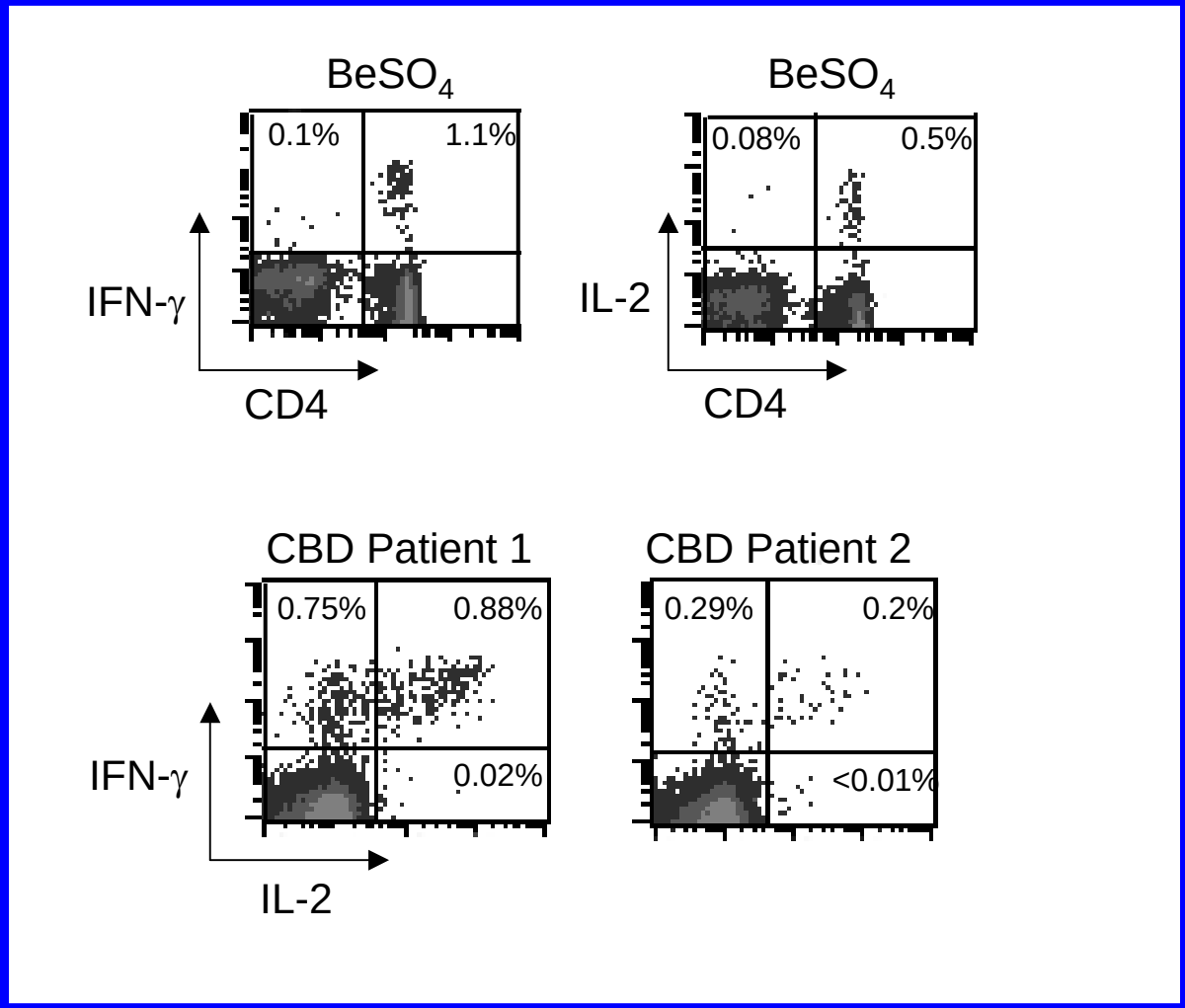
IFN-γ Versus IL-2 Expression by Be-Specific $CD4^+$ T Cells



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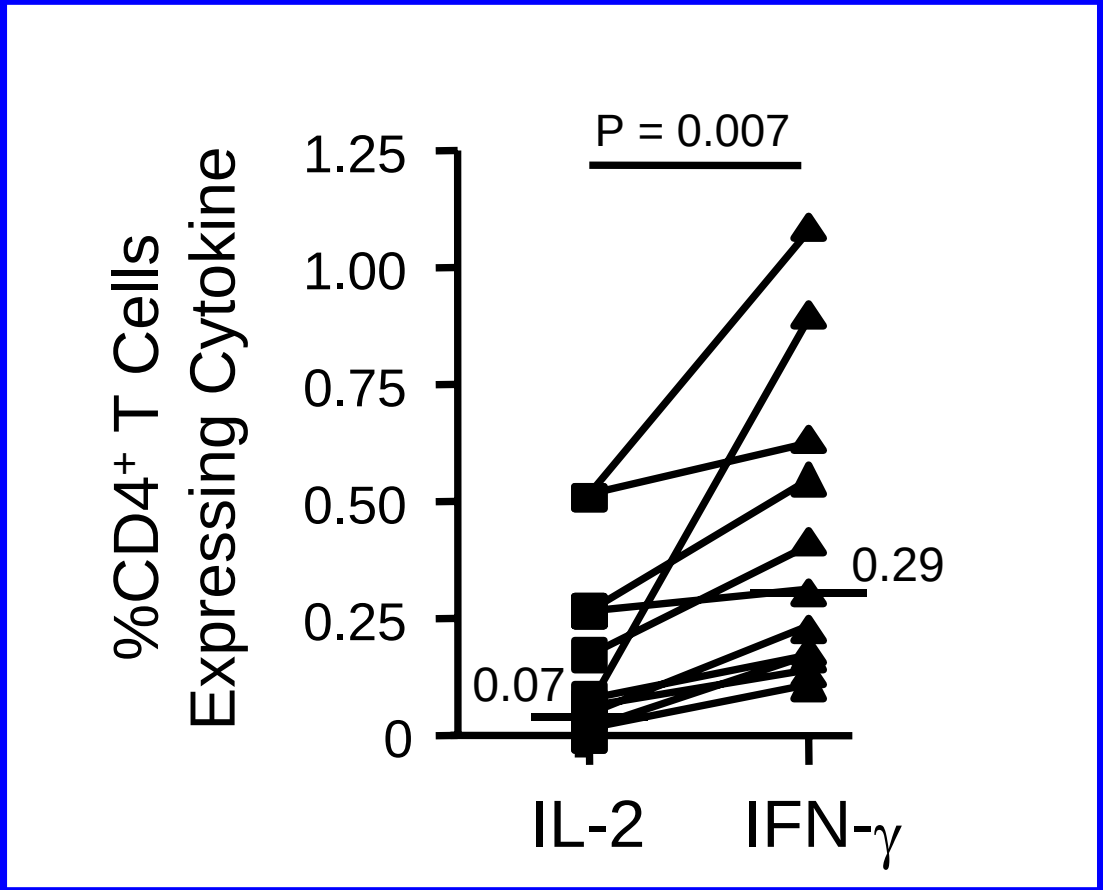

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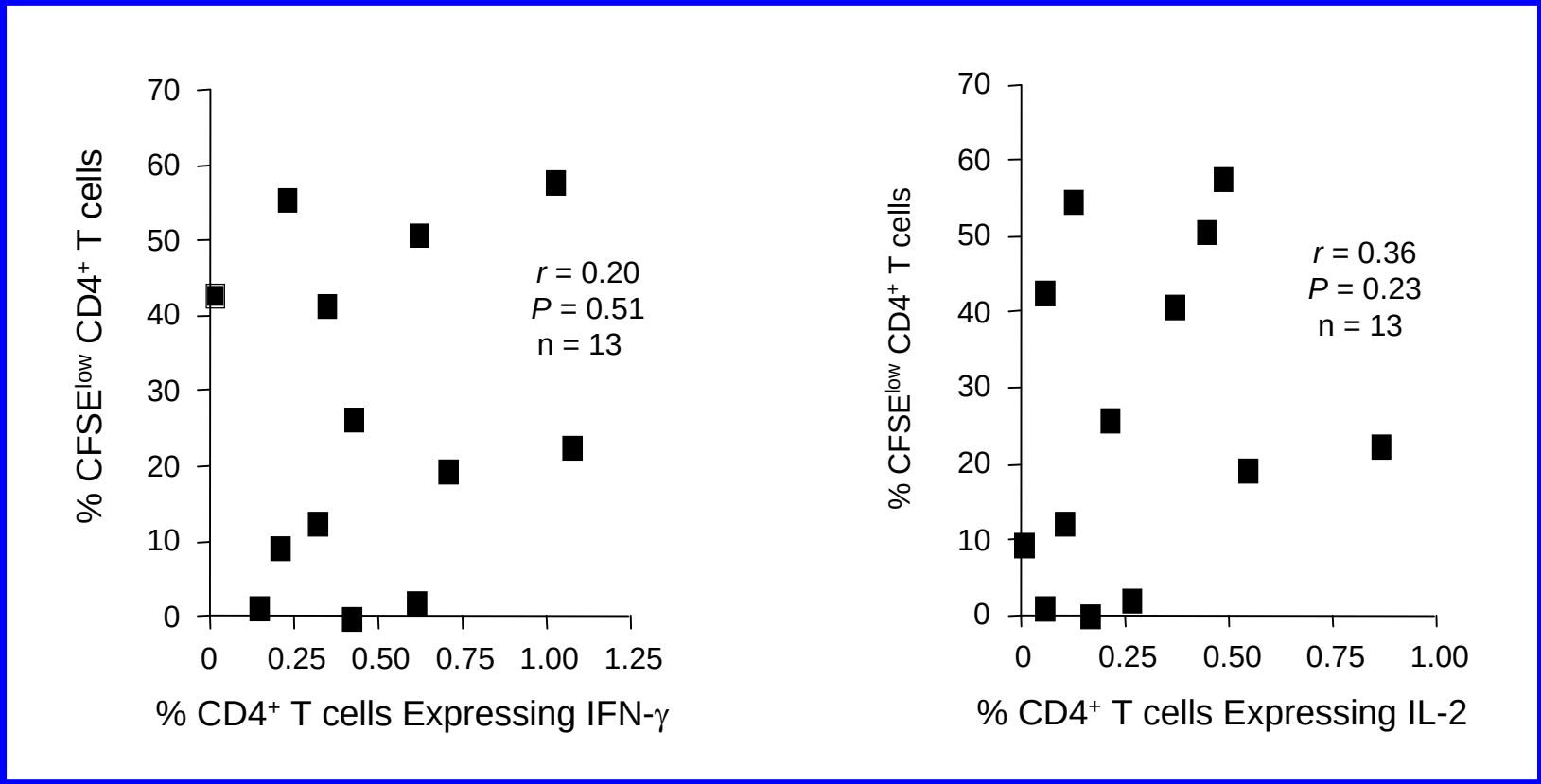
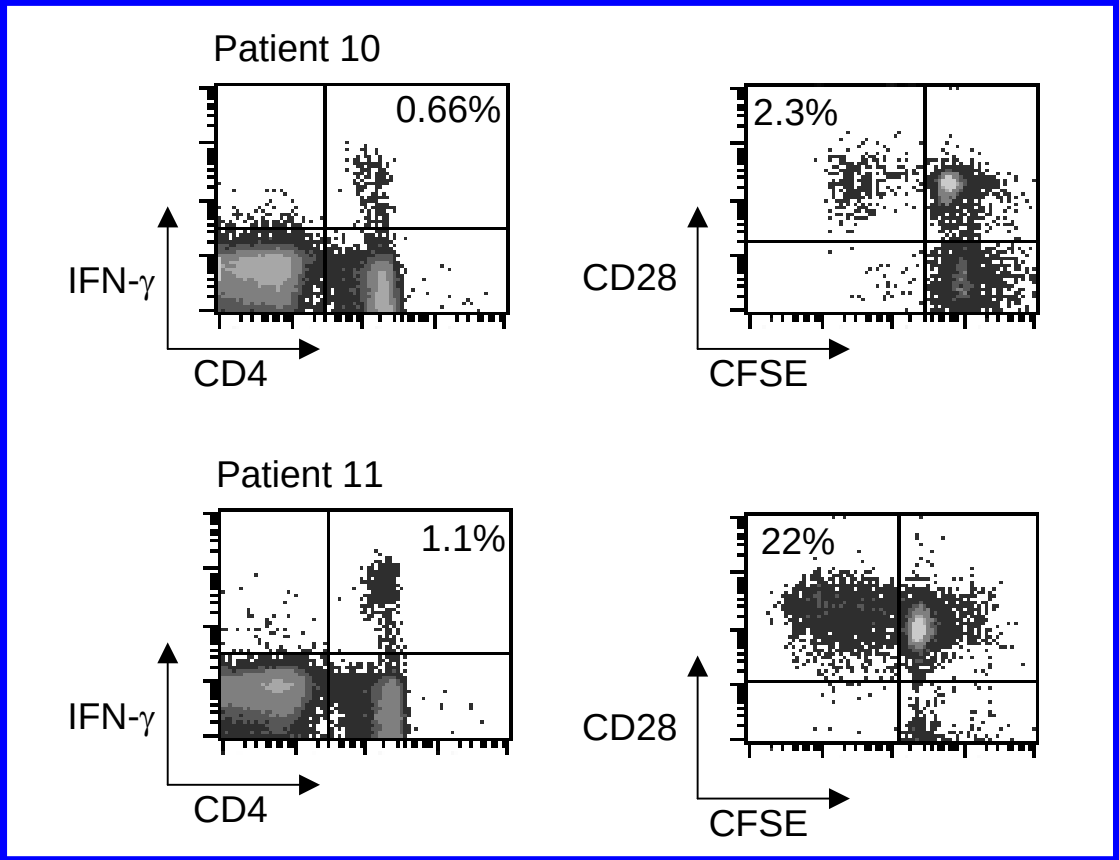
Correlation of Clinical Variables and Frequency of Beryllium-Specific T Cells in Blood

	r	P
Beryllium exposure (yrs)	0.02	0.89
Disease duration (yrs)	0.26	0.15
BAL parameters		
BAL WBC count	**0.41**	**0.004**
BAL lymphocyte count	**0.45**	**0.002**
Exercise physiology		
PaO2, rest, mm Hg	0.09	0.62
PaO2, max, mm Hg	0.05	0.76
(A-a)O2 rest, mm Hg	-0.05	0.78
(A-a)O2 max, mm Hg	0.004	0.98

Lack of Correlation Between Beryllium-Induced Proliferation and Cytokine Secretion



Variability in Beryllium-Induced Proliferation Between CBD Patients

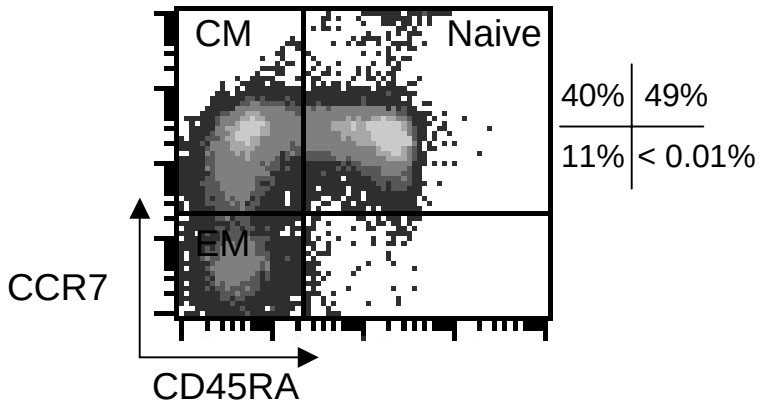


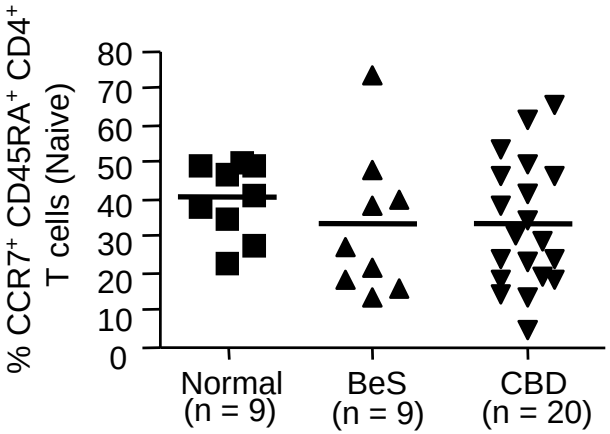
Memory $CD4^+$ T Cells

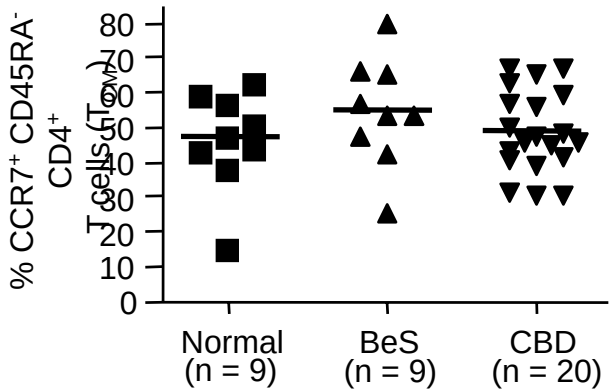
- There are at least 2 subsets of memory T cells
  - Effector memory cells which lack lymph node homing receptors, CD62L and CCR7
  - Central memory cells which express both CD62L and CCR7 but lack immediate effector activity
  - Once stimulated, central memory cells can differentiate into effector memory cells

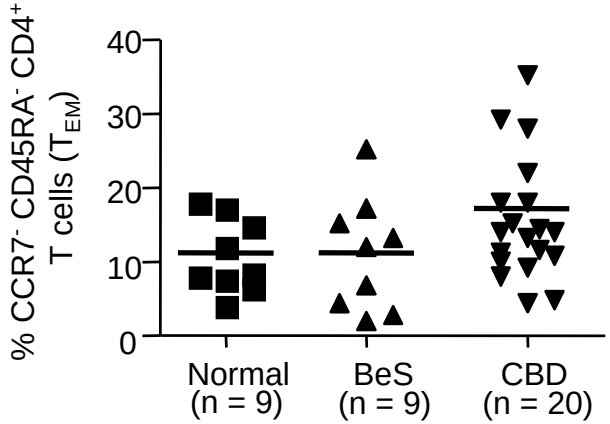
Memory movie

Characterization of the Total Memory CD4+ T Cell Populations in Blood

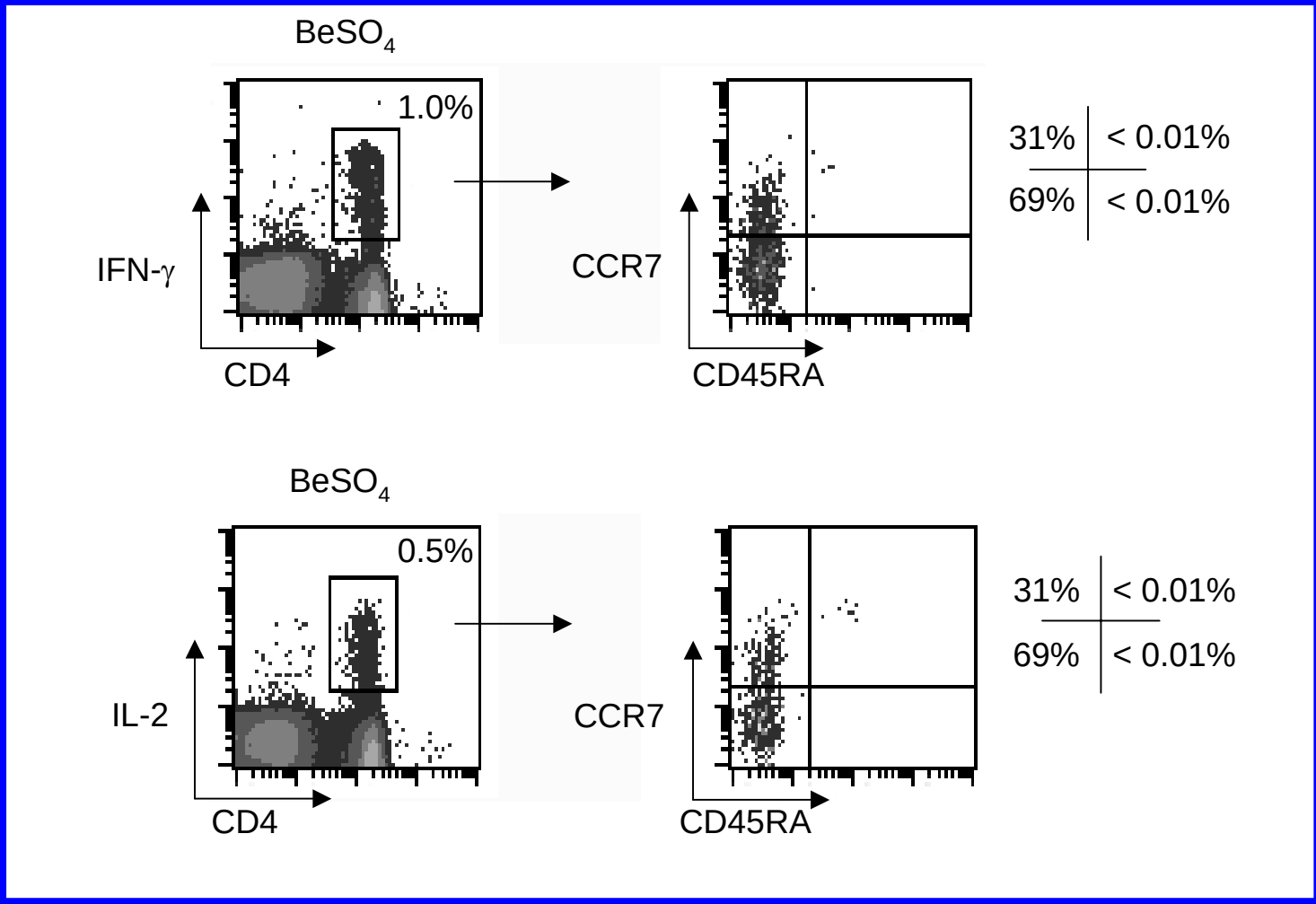




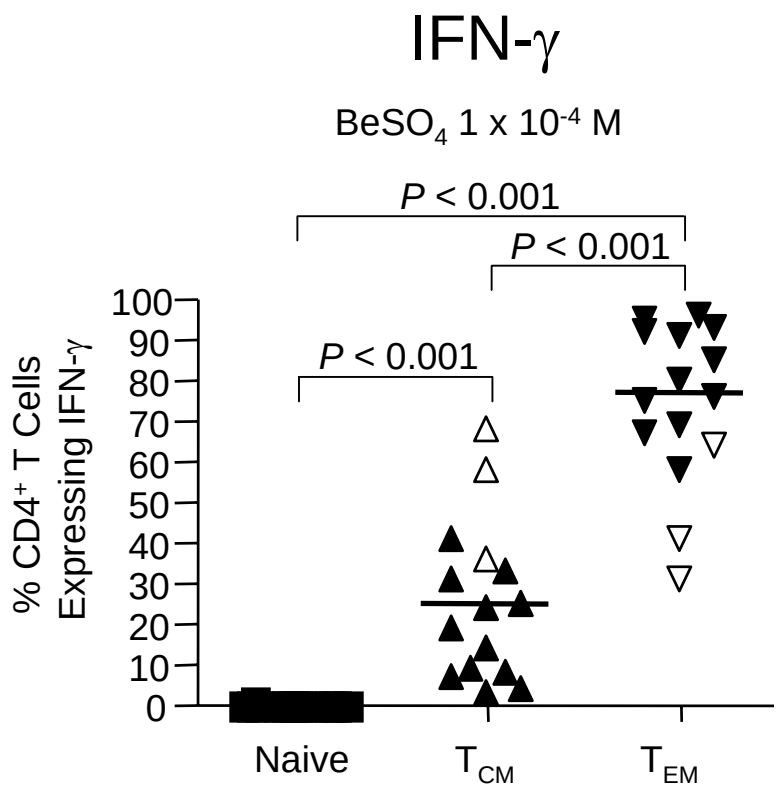


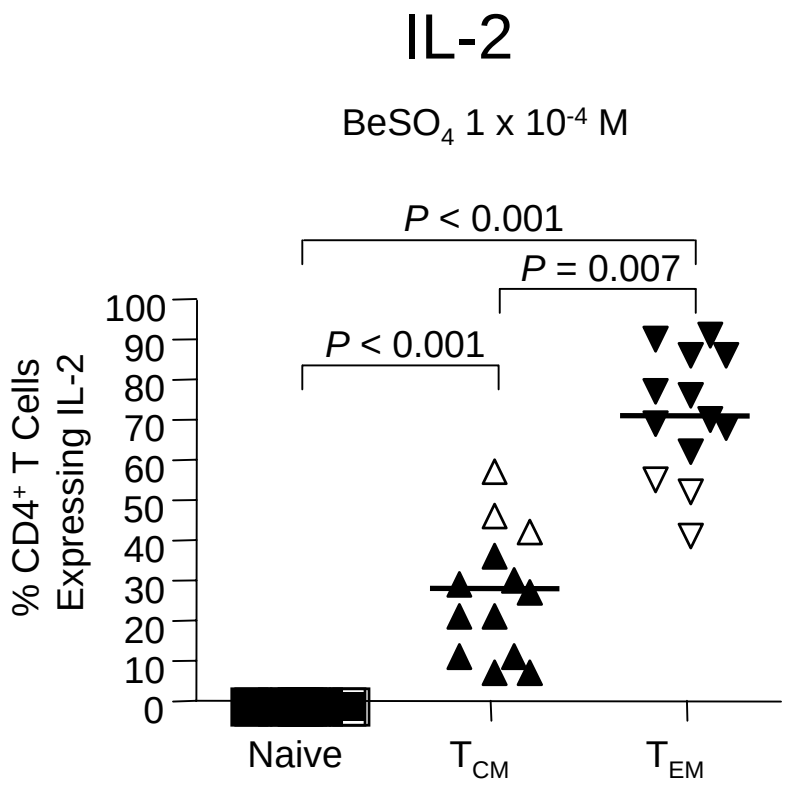


Be-Specific CD4$^+$ T Cells in Blood of CBD Patients Express an Effector Memory Phenotype

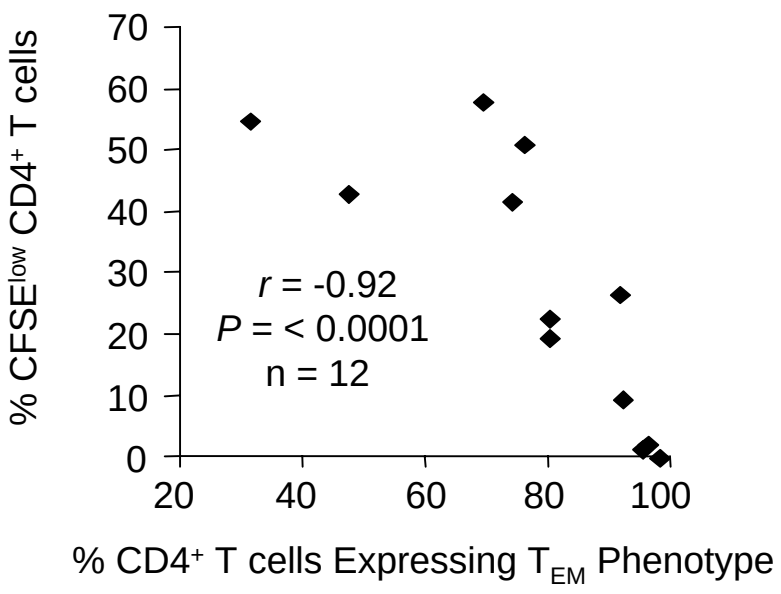


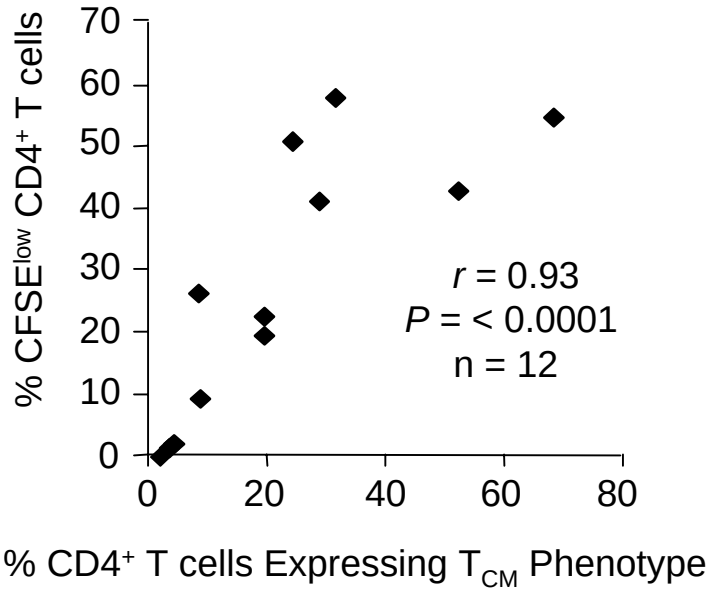
Memory Maturation Phenotype of Beryllium-Specific CD4$^+$ T Cells



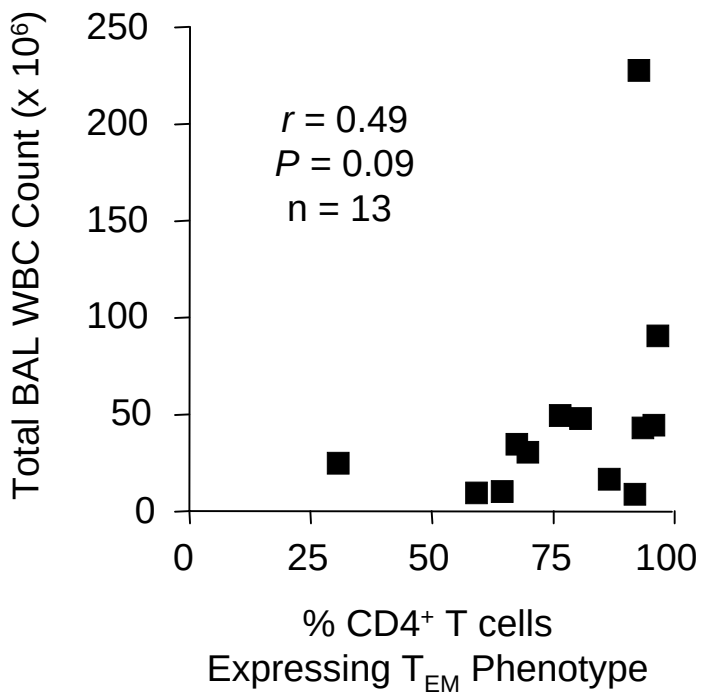


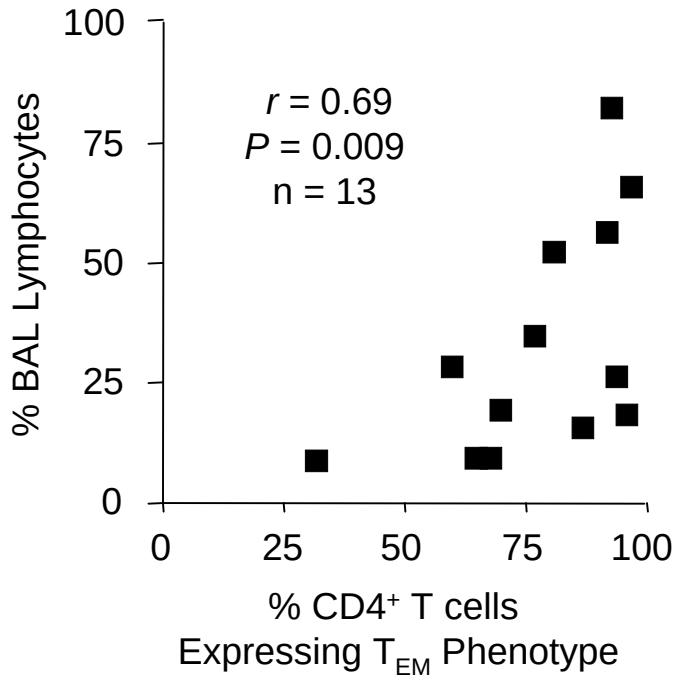
Correlation of Beryllium-Induced Proliferation and TCM Phenotype





Relationship of Maturation Phenotype of Blood T cells to Lung Inflammation





Conclusions

- Compared to BeS subjects, patients with CBD have more Be-specific T cells in blood
- ELISPOT analysis may be used to differentiate between BeS and CBD without the need for more invasive tests
- Majority of beryllium-specific $CD4^+$ T cells in blood express an effector memory phenotype
- Proliferation is closely tied to the maturation state of the beryllium-specific T cell
- Functional capability of T cells is determined by the proportion of memory cell subsets, which may reflect target organ involvement

Acknowledgments

- UCHSC
  - Greg Pott
  - Allison Martin
  - Andrew Sullivan
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