Monitoring of Naturally Acquired and Vaccine-Induced SARS-CoV-2-Specific Cellular Immune Responses

Amir Ameri[1], Dilek Inekci[1], Bjarke Endel Hansen[1], Matilda Weywadt[1], and Liselotte Brix[1]

[1]Immudex, Copenhagen, Denmark

Introduction

The global outbreak of the COVID-19 pandemic has emphasized the importance of immune monitoring technologies to reveal the dynamics of the immunological response in naturally acquired immunity, vaccination, and hybrid immunity. Immune monitoring technologies can help provide guidance to clinicians and health authorities about the type, magnitude, and duration of cellular immunity and thus improve the decision-making about future vaccine programs and roll out of booster doses.

Our aim was to develop SARS-CoV-2-specific immune monitoring assays based on the Dextramer® technology to detect and characterize virus-specific T-cell responses. The applicability of the Dextramer® assay for immune monitoring in SARS-CoV-2 was demonstrated by a longitudinal case study on an individual with hybrid immunity.

Generation of SARS-CoV-2 MHC I Dextramer® Panels

SARS-CoV-2 Dextramer® Spike Panel

Allele	Peptide	Antigen
A*0101	LTDEMIAQY	S
A*0101	WTAGAAAYY	S
A*0201	YLQPRTFLL	S
A*0201	NLNESLIDL	S
A*0201	FIAGLIAIV	S
A*0301	KCYGVSPTK	S
A*0301	GVYFASTEK	S
A*1101	RLFRKSNLK	S
A*1101	KCYGVSPTK	S
A*1101	GVYFASTEK	S
A*2402	QYIKWPWYI	S
A*2402	NYNYLYRLF	S
B*0702	SPRRARSVA	S
B*0702	APHGVVFL	S
B*3501	QPTESIVRF	S
B*3501	LPFNDGVYF	S
B*3501	IPFAMQMAY	S

SARS-CoV-2 Dextramer® Non-Spike Panel

Allele	Peptide	Antigen
A*0201	LLLDRLNQL	N
A*0301	KTFPPTEPK	N
A*1101	ATEGALNTPK	N
A*1101	KTFPPTEPK	N
B*0702	KPRQKRTAT	N
B*0702	SPRWYFYYL	N
A*0101	FTSDYYQLY	ORF3a
A*0201	LLYDANYFL	ORF3a
A*2402	VYFLQSINF	ORF3a
A*0101	CTDDNALAYY	ORF1ab
A*0101	TTDPSFLGRY	ORF1ab
A*0201	ALWEIQQVV	ORF1ab
A*0301	KTIQPRVEK	ORF1ab
A*0301	VVYRGTTTYK	ORF1ab
A*1101	ASMPTTIAK	ORF1ab
A*2402	VYIGDPAQL	ORF1ab
B*0702	IPRRNVATL	ORF1ab

The developed SARS-CoV-2 MHC class I Dextramer® panels cover alleles A*0101, A*0201, A*0301, A*1101, A*2402, B*0702, B*3501 and B*4402 complexed to immunodominant epitopes from Spike and Non-Spike (Nucleocapsid, ORF1ab and ORF3a) proteins of the SARS-CoV-2 reference strain (Lineage B, NCBI RefSeq reference genome NC_045512) with a predicted coverage of 87% and 94% of the US and EU population, respectively, (population coverage was predicted using the IEDB.org tool at http://tools.iedb.org/population/).

Screening of a Vaccinated Convalescent Individual

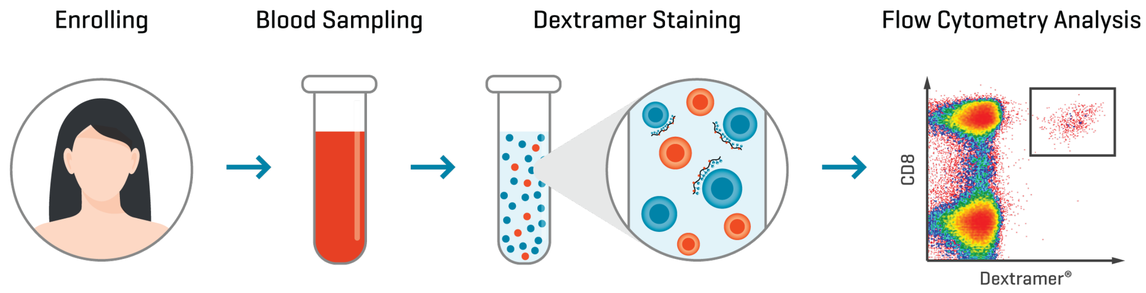

Figure 1. Schematic overview of the experimental workflow to Dextramer® staining of the CD8⁺ T-cell response to SARS-CoV-2. To evaluate the clinical performance of the assay, consecutive PBMC samples were collected assay to determine the frequency of SARS-CoV-2-reactive CD8⁺ T cells using the SARS-CoV-2 MHC I Dextramer® panels:

- Before the pandemic was used as a reference.
- After 1st and 2nd vaccination
- After infection post vaccination

Staining procedure:

Briefly, PBMCs were stained in a tube containing Dextramer® reagents from the Spike (PE labelled) and Non-Spike (APC labelled) panels. In a second tube cells were labelled with Dextramer® reagents consisting of same HLA alleles displaying influenza, EBV or CMV epitopes (PE labelled) as well as negative control Dextramer® reagents (APC labelled). Samples were analyzed using flow cytometry.

Dextramer® assay reveals changes in magnitude and kinetics of SARS-COV-2 specific CD8⁺ T-cell immunity

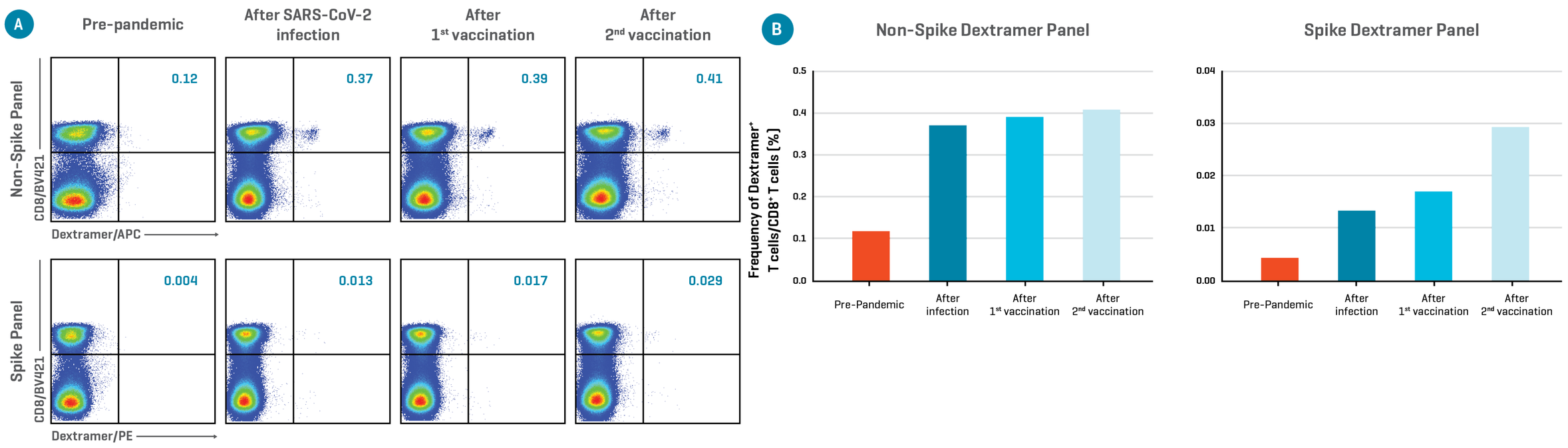

Figure 2. A SARS-CoV-2 CD8+ T cell response was detected in all samples after infection and vaccination. A) Flow cytometry plots showing SARS-CoV-2-specific CD8⁺ T cells reactive with Spike and Non-Spike epitopes detected in PBMCs from a vaccinated convalescent donor. The frequencies are here defined as % frequency of SARS-COV-2-specific CD8⁺ T cells of total count of CD8⁺ T cells. **B)** Bar plots summarizing the change in the frequencies of Spike- and Non-Spike-specific CD8⁺ T cells across time points. A CD8⁺ T-cell response was detected for all time points after infection and vaccination. There was no detectable response against either Spike or Non-Spike in the pre-pandemic sample. CD8+ T cells specific to the Spike epitopes increase after infection, but their numbers increase even further after each dose of vaccine. This could indicate that vaccination boosted the initial immune response to a higher level than that following the naturally acquired infection, in agreement with studies that have reported convalescent individuals show a greater response to the vaccine compared to naïve individuals. The quantity of Spike-specific T cells has been reported to vary depending on factors such as disease severity and time after infection or vaccination. Different subpopulations of T cells can also differ in longevity, so the magnitude and kinetics of the T cell response needs further investigation. CD8+ T cells specific to Non-Spike epitopes were elevated to a similar level in samples after infection, 1st and 2nd vaccination, compared to the pre-pandemic sample. This may be because the vaccine is based on the Spike protein, hence the Non-Spike response is not boosted by the vaccine. There was no detectable CD8+ T-cell response against Spike or Non-Spike in the pre-pandemic sample, which suggests the absence of pre-existing immunity towards COVID-19 in this individual. SARS-CoV-2 epitopes may be cross-reactive with common cold coronaviruses19,20, raising the intriguing possibility that prior infection with the common cold could lead to cross-reactive memory T cells that may potentially provide a level of protection against SARS-CoV-2.

Conclusion

- We developed SARS-CoV-2 Dextramer® Panels to monitor the dynamics of SARS-CoV-2-specific CD8+ T cell responses over time.
- We analyzed PBMC samples collected from an individual pre-pandemic, post-infection and post-vaccination and demonstrated that Spike and Non-spike-specific CD8+ T-cell responses exhibited different patterns of magnitude & kinetics.
- SARS-CoV-2 Dextramer® panels can monitor long-term cellular immunity following infection or vaccination.
- This approach can be used to evaluate new vaccine candidates and support booster vaccination decision making by identifying sub-populations of individuals with low or declining immunity

Perspective: Intelligent monitoring of immunity by the SARS-CoV-2 Dextramer® assay

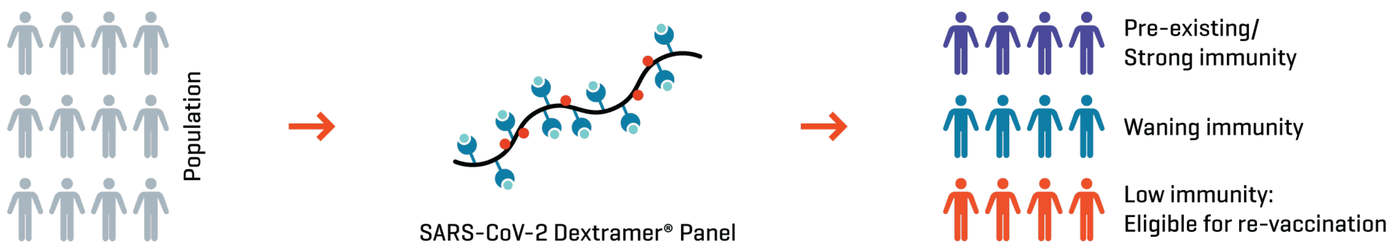

Figure 3. Proposed model for using the Dextramer® assay for population stratification based on the SARS-CoV-2 immunity status. A detailed understanding of the long-term cellular immunity in COVID-19 is essential to guide future vaccination strategies and help to avoid a recurrence of the pandemic, as new variants emerge.

Analysis of populations by Dextramer® Panel can reveal the immune status and stratify the population into sub-populations based on immunity towards SARS-CoV-2. Sub-populations showing low immunity are eligible for revaccination. Grey color symbolizes unknown status of immunity in the population.

IMMUDEX®