454 Pyro-sequencing of T Cell Receptors Targeting Pancreatic Islets

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Purpose: The ultimate goal is to directly isolate T cell receptor (TCR) alpha and beta chain sequences from T cells infiltrating pancreatic islets of patients having type 1 diabetes (T1D) and to identify their combinations responding to islet antigens. Our first goal was to establish methodology that allows us to precisely identify "frequent" TCR sequences from limited amount of cells and tissues. Using the optimized protocol, we determined TCR sequences frequently detected in two samples from patients having type 1 diabetes.

Methods: The first RNA sample was collected from peripheral blood mononuclear cells of a T1D patient having DR4/13. We extracted RNA from the 3000 cells sorted with the gating of CD4+ and CFSE-low after a week of culture with the insulin B:9-23 peptide in the presence of IL-7 and anti-FasL Ab. The second RNA sample was obtained from the 2 x 2 inches x 20 μm of histology section generated from nPOD #6052 pancreas head. We amplified TCR alpha and beta chain DNA fragments from the RNA samples by 5'RACE PCR and ran PCR products on the 454 GSJR sequencer. To evaluate reproducibility, all the PCR products were marked by multi-identifier adaptor primers, which allow us to distinguish sequences obtained from individual PCR reactions, and were run on the 454 GSJR simultaneously. All sequences were analyzed using the IMGT-HighV-QUEST algorithm.

Summary of Results: We obtained 60,428 alpha chain and 83,224 beta chain in-framed sequences from 24 PCR reaction products generated from the 3,000 CFSE-diluted CD4 T cells. As shown in Table 1 and 2, we obtained multiple TCR sequences that are consistently frequent in multiple PCR reactions. Reproducibility among the multiple PCR reactions for both alpha and beta chains are convincing enough to identify frequent TCR chain sequences (mean R2 = 0.75 and 0.85 for alpha and beta chains respectively). Of interest, 3 out of the top 10 frequent alpha chains use the identical TRAV and TRAJ segments (TRAV14 and TRAJ5) with various junction sequences. We are currently generating Jurkat cell lines retrovirally expressing multiple combinations of these frequent alpha and beta chains to test them for response to the insulin B:9-23 peptide. Table 3 shows all the alpha chain sequences detected in 31 PCR reactions generated from the nPOD 6052 pancreatic head histology section. Although we obtained total 102,616 sequences, only 1-3 unique junction sequences were found in the individual PCR reactions. However, some of them are repeatedly detected by multiple reactions. Such sequences may frequently exist in the islets.

Conclusions: We have established the pyro-sequencing method to detect TCR sequences from the small amount of samples. Function analysis by generating T cell lines expressing frequent TCR sequences will confirm that such TCRs are truly islet-reactive and contribute to targeting pancreatic beta cells.

(Tables on next page)

Individual columns represent PCR reactions. Frequencies of TCR sequences were shown in percentage.
Dark green : >10%, Moderate green: 5-10%, Light green: 1-5%, White: not detected

Table 1: Frequent alpha chains detected in B:9-23-reactive CD4 T cells in the peripheral blood by 454 TCR sequencing with 24 PCR reactions

Vgene	Junction	Jgene	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
TRAV16	CALRFGSSNTGKLIF	TRAJ37																								
TRAV8-2	CVVQRITITMTCAPSDNNNDMRF	TRAJ43																								
TRAV12-1	CVVNKGTNAGKSTF	TRAJ27																								
TRAV23/DV6	CAASGGTGGFKTIF	TRAJ9																								
TRAV38-1	CAFMSPYGGATNKLIF	TRAJ32																								
TRAV14/DV4	CAMRPMDTGRRALTF	TRAJ5																								
TRAV14/DV4	CAMRGVDTGRRALTF	TRAJ5																								
TRAV14/DV4	CAMIPMDTGRRALTF	TRAJ5																								
TRAV21	CAVKFNKFYF	TRAJ21																								
TRAV6	CALVRSTDKLIF	TRAJ34																								

Table 2: Frequent beta chains detected in B:9-23-reactive CD4 T cells in the peripheral blood by 454 TCR sequencing with 22 PCR reactions

Vgene	Junction	Jgene	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
TRBV19	CASSIVGRAVDEQFF	TRBJ2-1																						
TRBV10-2	CASSERAGGSTDTQYF	TRBJ2-3																						
TRBV7-9	CASSSIWGEETQYF	TRBJ2-5																						
TRBV6-2/6-3	CASEGAGGNEQFF	TRBJ2-1																						
TRBV6-2/6-3	CASSDSSGGAGTDTQYF	TRBJ2-3																						
TRBV28	CASSGTGGGFSYTF	TRBJ1-2																						
TRBV24-1	CATSDSSGGRETQYF	TRBJ2-5																						
TRBV28	CASNSDSTGSWGQPQHF	TRBJ1-5																						
TRBV5-1	CASSETGTGKPDTQYF	TRBJ2-3																						
TRBV10-3	CAISESISPEQFF	TRBJ2-1																						
TRBV19	CASSIHGTRNTEAFF	TRBJ1-1																						
TRBV5-1	CASSPKSTSGGDNEQFF	TRBJ2-1																						

Table 3: Alpha chain sequences detected in the nPOD6052 pancreas head histology section by 454 TCR sequencing with 31 PCR reactions

Vgene	Junction	Jgene	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
TRAV12-2	CAVGGAAGNKLTF	TRAJ17																															
TRAV12-2	CAVTMGSNDYKLSF	TRAJ20																															
TRAV12-3	CAMSARGGSYIPTF	TRAJ6																															
TRAV13-1	CAATPGGYNKLIF	TRAJ4																															
TRAV13-2	CAESSSYKLIF	TRAJ12																															
TRAV13-2	CAENPSGDGGYNKLIF	TRAJ4																															
TRAV13-2	CAENSGMNYGGSQGNLIF	TRAJ42																															
TRAV17	CATDASVTPLVF	TRAJ29																															
TRAV19	CALSEARSNDYKLSF	TRAJ20																															
TRAV19	CALSELSGATNKLIF	TRAJ32																															
TRAV19	CALSEAGNYGGSQGNLIF	TRAJ42																															
TRAV19	CALSEAQGGRRALTF	TRAJ5																															
TRAV21	CAVTGGNKLTF	TRAJ10																															
TRAV21	CASDSGSARQLTF	TRAJ22																															
TRAV21	CAVKPPVGGGKLIF	TRAJ23																															
TRAV21	CAVKSNSGNTPLVF	TRAJ29																															
TRAV21	CAAYSTGKLIF	TRAJ37																															
TRAV22	CAALYGNKLVF	TRAJ47																															
TRAV23/DV6	CAATPGRSGGYQKVTF	TRAJ13																															
TRAV26-1	CIVRVENQGGKLIF	TRAJ23																															
TRAV26-2	CILGENQAGTALIF	TRAJ15																															
TRAV26-2	CILNSGNTPLVF	TRAJ29																															
TRAV27	CAGKDTNAGKSTF	TRAJ27																															
TRAV38-1	CAFRVDSSYKLIF	TRAJ12																															
TRAV38-2	CAYFSGTYKYIF	TRAJ40																															
TRAV38-2	CAYRSGSNNDMRF	TRAJ43																															
TRAV4	CLVGDPSTGGAGNMLTF	TRAJ39																															
TRAV4	CLVGPLMFSGGYNKLIF	TRAJ4																															
TRAV41	CAVSFGNEKLTF	TRAJ48																															
TRAV41	CAEFYF	TRAJ49																															
TRAV6	CALDPSGGSYIPTF	TRAJ6																															
TRAV6	CAVGDPSFGNEKLTF	TRAJ48																															
TRAV8-6	CAPSPGGYNKLIF	TRAJ4																															
TRAV8-6	CAVSDMGTYKYIF	TRAJ40																															
TRAV9-2	CALSPDYKLSF	TRAJ20																															
TRAV9-2	CALSDRANAGKSTF	TRAJ27																															
TRAV9-2	CALRGSSGYELNF	TRAJ41																															