

To the IDS President

To the IDS Council

To all IDS Members

October 16, 2008

Dear Colleagues,

This letter is to introduce the initiatives that the T Cell Workshop (TCW) Committee is launching for the next few years.

Following the IDS-9 held in Miami in November 2007, Mark Peakman and Bart Roep handed over the relay to a new TCW Steering Committee. This is also an occasion to thank Drs. Peakman and Roep for their efforts throughout these years, and for their continuing support and expert advice for the new initiatives.

Upon assuming this responsibility, we quickly realized the time and effort needed to foster and facilitate these activities. We therefore decided to have our initiatives coordinated by a Steering Committee rather than by a single Chairman. Within this Committee of nine people, a rotating Coordinator will be appointed every 6 months to lead and harmonize the group's efforts. Dr. Corrado Cilio will be the Coordinator from October 2008 until May 2009.

The Steering Committee has enjoyed continuous and frequent exchange of information during the past ten months and has attempted plan a path to be followed for several years ahead. The five objectives of the TCW activities are established by the IDS statute, and will be the focus of our new initiatives. As these aims are all interrelated and may be approached in parallel, they will nevertheless be addressed according to a ranked order of priority. Outlined below is the relative priority to be assigned to each of these objectives, the pending issues encountered at each step, and how we propose to address them:

1. Definition and use of standard protocols: the first protocols to be standardized should be those shared by any T cell assay, i.e., PBMC preparation and freezing/thawing. While a lot of work has been performed in the past, no consensus has been reached. This is because most studies have been performed by looking at recall Ags not related to diabetes, the response to which may follow different rules. Moreover, the applicability of a freezing protocol has been traditionally addressed focusing on a specific T cell assay format rather than on multiple ones. Defining a common protocol for PBMC freezing and thawing is therefore our top priority. This is because subsequent use of frozen samples would greatly facilitate multi-centre studies; and because comparison of fresh vs. frozen T cells is another key objective of the TCW activities (see point 2).

Proposal: previous work on the subject has been reviewed to avoid unnecessary replication, and two critical parameters which deserve initial testing have been identified, i.e., the use of cold vs. warm freezing media. We have therefore prepared a study outline (the IDS TCW Freezing Study I) where these two parameters are evaluated, and the performance of fresh vs. frozen PBMCs compared. To avoid conclusions that may apply only to a specific assay, each Laboratory will use its own T cell assay on T1D vs. healthy subject samples. Participation to this initial pilot study will be limited to few Laboratories within the T Cell Workshop Steering Committee. These Laboratories have the task of deciding between the “warm” and the “cold” freezing protocols is the best performing (i.e., the one which leads to better cell recovery, viability, and reproducibility as compared to fresh samples). The chosen protocol will subsequently be used as a “gold standard”, against which novel freezing procedures will be tested. These further freezing studies will be open to all IDS Laboratories willing to participate. Development of such standardized procedures would be of interest to most Labs, and with a large number of participants, the effort within each Lab could be limited to few samples to reach sufficient statistical power. The deliverable would be an IDS consensus statement on recommended freezing/thawing procedures.

2. Comparison of fresh vs. frozen T cells (an assay-dependent issue): although assay-dependent, this issue is closely related to that of defining a standard freezing protocol.

Proposal: these two points will therefore be addressed in parallel, as outlined above. As multiple assay platforms are considered, conclusions will be more easily generalized. We may either identify a freezing protocol suitable for all T cell assays; or different procedures may apply depending on the assay. The second deliverable could be the identification of T cell assays that work on frozen PBMCs.

3. Validation of existing T cell epitope specificities: several T cell epitopes have been described in the literature using several approaches, providing more or less stringent evidence for relevance to T1D. Having an independent, multi-centre validation of these epitopes would constitute a formidable resource for the research community.

Proposal: as a first step, the recent epitope synopsis of DiLorenzo et al. (Clin.Exp.Imunol., 148:1-16, 2007) will be made available on the new IDS website in an interactive form, which will be periodically updated by the Author. Submission of newly published epitopes will also be possible. A second, ambitious proposal called TDEVI (Tetramer-Directed Epitope Validation Initiative) has been promoted by the Benaroya Research Institute (BRI) in Seattle and subscribed by this Steering Committee. We propose to launch a multi-centre effort for validating CD4⁺ and CD8⁺ T cell epitopes reported in the literature. The BRI will coordinate with NIAID the synthesis of these peptides as well as that of HLA Class I and Class II tetramers specific for selected alleles and epitopes. These reagents will be distributed to participating Laboratories and used for reactivity testing. Assays will be performed on PBMC samples from locally recruited T1D and healthy subjects. Frozen samples may also be exchanged among Labs. Note that these assays are not proposed for clinical application (i.e., for diagnostic purposes or for following disease progression) at this stage, but exclusively as methods suitable for epitope validation purposes. Rather, participating laboratories will be

encouraged to test their own T cell assays in parallel (see point 5). A small pilot, proof-of-concept study will be launched by the end of the year, initially involving the Steering Committee Laboratories and eventually (i.e., by the end of 2009) all IDS Laboratories willing to participate. The deliverable would be a definitive, independently validated epitope panel (i.e., epitopes recognized by T1D but not by healthy PBMCs), which would be most valuable for subsequent assay standardization and clinical application efforts.

4. Validation, comparison and provision of shared reagents: this point is critical to facilitate assay comparison. At this stage, shared reagents include epitope peptides, recombinant antigen proteins and tetramers. The definition may also be extended to frozen PBMC samples.

Proposal: production and distribution of these reagents is planned in both the IDS TCW Freezing Study I and TDEVI initiatives. The deliverables would be validation results independent of reagent variability; and blinding of these reagents to draw more robust conclusions.

5. Standardization of assays: the validation and dissemination of T cell assays suitable for clinical applications are ultimately the most important goals.

Proposal: in parallel with the epitope discovery component of the TDEVI initiative, a comparison of different CD4⁺ and CD8⁺ T cell assays will also be undertaken. Along with the epitope validation assays, participating Labs will perform their own local T cell assays. The TDEVI initiative will therefore provide information not only on epitopes relevant to T1D, but also on the relative performance of different T cell assays. In a second phase, the protocols of the best performing assays will be distributed and subjected to further multi-centre, blind-coded validation.

Thus, the three main goals of the TCW for the next three years would follow the objectives outlined above:

Goal #1: to define standard freezing/thawing protocols to be used for T cell assays

Goal #2: to validate known T cell epitopes through the TDEVI multi-centre T cell initiative

Goal #3: to validate relevant T cell assays, first through the TDEVI initiative; and later through testing of the selected assays by multiple Laboratories.

The Steering Committee will further develop and revise these initiatives via e-mail exchange and monthly conference calls. Communication between the Committee and the wider IDS community will occur monthly by circulating and posting an e-mail newsletter on the IDS website and by encouraging the input and participation of all interested IDS investigators in the development of such initiatives.

While these efforts currently rely on the willing and interest of participating Laboratories, appropriate funding to support these initiatives will also be crucial. By gathering additional preliminary data, the Freezing Study I and TDEVI pilot studies will strengthen the potential of our proposals. In parallel, we will continue to explore the most appropriate funding models to

bring these initiatives to the next step, which is that of larger-scale studies and of wider involvement of the whole IDS community.

We hope that these proposals will be of immediate interest to the IDS Community. We look forward to your input and participation to enhance our further progress and achieve our goals.

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