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Letter to the editor

Immune Functions of Peptides from Self-Protein Versus Foreign Viral Protein

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Introduction

Amino acid (AA) sequences found in human protein C3d (Complement fragment) are predicted by computer algorithm "EpiMatrix" to be MHC class II-binding T-cell epitopes [1]. Synthetic peptides with these sequences added to cultures of normal WBCs stimulate mitosis of $CD4^+$ T-cells producing interferon gamma (IFNγ) [2], although their functional phenotypes have not yet been determined. A different mixture of 28 synthetic peptides (18mer T-cell epitopes overlapping by 11 AAs spanning the entire hepatitis C virus (HCV) core protein) [3,4] when added to PBMCs of *chronic* HCV patients, IFNγ production by $CD25^+$ Treg cells was *not observed* but $CD25^-$ T conventional (Tconv) cells retained the ability to produce IFNγ [5]. Naturally occurring Tregs [thymus-derived regulatory T cells] are an important cell type in the maintenance of peripheral tolerance [6-8]. In humans, such Tregs are present within the CD4+/$CD25^{hi}$ cell population, expressing transcription factor FoxP3, which controls their immunosuppression activity. De Groot [9] added C3d peptides to WBCs of normal *healthy* donors, 3 different peptides of complement protein C3d [p223-246; p248-266; p269-286: (18-24 AAs)], each prepared with peptides blocked at their N/C-terminals to protect them from terminal AA proteases), alone or in a pool. These C3d peptides are considered as derived from *self-antigen* (C3).

Methods of IFNγ detection differed in the two laboratories: flow cytometry (FACS) *vs* ELISpot [2,5,9]. Incubation times also were unequal. Incubation with HCV peptides was 5 days; while with C3d peptides was 10 days [2,5,9]. Shorter and longer times may be explored in future. Li et al. followed one of the classical intracellular cytokine detection procedures: they incubated HCV peptides with chronic hepatitis patient PBMCs for 5 days; cultures were harvested and re-stimulated with anti-CD3 and IL2 for 6 hours in the presences of Brefeldin A, followed by surface/intracellular staining. WBCs in floating (buffy coat) layer on top of the Ficoll gradient from "normal" healthy donor blood (cryo-preserved in liquid nitrogen) after thawing and centrifugation [2,9]. Most RBCs and ruptured-cell membrane with cytoplasm contents pelleted; floating WBCs were induced with C3d peptides. On day 10, PBMCs were harvested and added (2.5×10^5 cells per well) to Mabtech® plates pre-coated with anti-IFNγ antibody, counted blind after 2 days by external consulting firm, Zell net.

The culture medium used for C3d [2,9] consisted of RPMI·1640 + Glutamax, with 10% human serum (blood type A/B), supplemented with fresh 1% L-glutamine, 1% 1M HEPES). IL2 (10 ng/ml), IL7 (20 U/ml) were added on day 1. About half of this medium was removed by suction on day 5 and replaced with fresh media + peptides as formulated on days 0 and 1. After 10 days incubation collects and count cells, prepares them for ELISpot assay [2,9].

In summary, Li showed that Treg from HCV patients produced little or no IFNγ, while Knopf et al. study showed that total PBMC from normal subjects produce IFNγ in response to (C3d).

Future Experiments I

In hepatitis patients infected with HCV, the CD25+ Treg cells do not produce IFNγ, even against a strong polyclonal stimulus by anti-CD3/IL2 for 6 hrs; BUT conventional T cells (CD25-) can produce IFNγ under the same condition [5]. We plan to collect additional phenotype data on $CD4^+$ T-cells stimulated by C3d peptides in healthy donors [2,9] to determine whether IFNγ-producing cells detected previously are conventional T cells (CD25-) or Tregs (CD25+) [10]. IFNγ responses may be considered as *anatomically associated with Ab (B-cell surface Ig) or TCR responses.* Surface-Ig or TCR molecules are specific to the inducing antigens.

Future experiments II

Assuming any differences in results between C3d and HCV models are not due to technical issues, such as incubation time or concentration of IL2 or IL7 in medium, if C3d peptide-stimulated healthy donor natural $CD4^+CD25^+$ Treg cells produce IFNγ, [2,9] We would like to consider the possibility of there being of an *inducer or inhibitor for IFNγ* production in each of the two systems: [2,9] vs [5]. We plan to *spike* this culture with HCV coat protein peptide antigen, to find out whether foreign antigen can inhibit Treg IFNγ response to C3d. We will culture HCV/patient PBMC with C3d, to find out whether self-antigen can reverse the non-IFNγ phenotype of HCV patient Treg cells. Such *dominance* assays will be conducted *in vitro*. A series of different incubation times (0, 5, 10, 15, 20 days or longer) of each peptide preparation alone (C3d or HCV core protein), since longer times of peptide exposure may allow slower growing cell populations to expand to detectable concentration and order of addition may be important to dominance of interactions. Will the detection of IFNγ be a dominance issue? If the C3d peptide addition dominates, then we probably conclude that Treg is non-proliferate *in vitro* when given *its cognate antigen* [11].

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