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Letter to the Editor

Competitive Antagonists of Adenosine Deaminase versus Enzymatic Inhibitors: Complementary Approaches Targeting Dipeptidyl Peptidase 4 (DP IV) and their Relation to MERS-CoV Infection

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DEAR EDITOR,

Because of concerns about its pandemic potential, therapeutic approaches to Middle East respiratory syndrome coronavirus (MERS-CoV) constitute pressing needs [1,2]. Van Doremalen and colleagues indentified, five amino acids within dipeptidyl peptidase 4 (DP IV) as critical for determining the species tropism for interaction with the receptor binding domain of MERS-CoV spike protein [3]. Based on previous reports, adenosine deaminase (ADA) might limit infection of mammalian cells by MERS-CoV through competition for the virus binding site on DP IV [4]. These findings could provide a basis for the development of pharmacological antagonists and antibodies [5-7] which can prevent DP IV- mediated entry of MERS-CoV into mammalian cells.

In our previous work we identified binding partners of DP IV including those that inhibit its enzymatic activity and provided a detailed analysis of the DP IV-ADA interaction [8]. In the present study, we present data from a comprehensive survey of the ability of several natural and pharmacological substances to inhibit ADA binding to DP IV (Table 1). These included agents from distinct molecular categories such as lectins, antisera and DP IV enzymatic inhibitors. As expected, specific antibodies efficiently blocked ADA binding to DP IV but several lectins were also highly potent in their inhibitory activity (Table 1). Of note, it was reported that HIV gp120 also binds to a region of DP IV similar to the binding site for MERS-CoV [9].

In addition to compounds that block non-catalytic moieties of DP IV, we identified a series of inhibitors of DP IV enzymatic activity that were shown to regulate immune responses *in vivo* [8, 10-14]. These inhibitors might modulate the pathogenesis of viral infection and serve as another class of potential therapeutics. Based on our work on the use of DP IV inhibitors as a treatment for autoimmune disease, DP IV inhibition could suppress the damaging aspects of the body's own antiviral immune response by modulating inflammation [8,10,13]. Reversible inhibitors of DP IV enzymatic activity suppress T cell proliferation and production of pro-inflammatory cytokines [8,13]. As we have shown, the DP IV inhibitor-mediated suppression acts in part through the induction of TGF-β1 production by effector T (Teff) cells at the site of the immune response within tissues. As a consequence of this action, the levels of latent TGF-β1 increased in tissue and plasma of mice treated with DP IV inhibitors [8,10]. Thus, TGF-β1 induction at the site of inflammation may be an additional therapeutic benefit of DP IV inhibitor treatment, as TGF-β1 has been shown to critically regulate immune responses in severe respiratory infections [15]. Importantly, injection of TGF-β delayed mortality and reduced viral titers of H5N1 influenza virus-infected mice while neutralization of TGF-β during H5N1 and pandemic 2009 H1N1 infection had opposing effects [15].

Taken together, the action of various ADA-DP IV binding antagonists as well as enzymatic inhibitors of DP IV should be explored in preclinical models as a prelude to determine their therapeutic effect in severe viral infections, including infection with MERS-CoV.

Regards,

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Table 1: Inhibition of ADA-DP IV binding[1]

Effectors binding	Inhibition of (% of max ± SD)
Antibodies	
Polyclonal goat-anti DP IV antibody	62.5 ± 12.3
Control serum (goat, mouse, sheep)	no effect
Ions	
(Ni^{2+}, Sr^{2+}, NH_4^+, Ba^{2+}, Ca^{2+}, Cd^{2+}, Mn^{2+}, $C0^{2+}$, Mg^{2+})	no effect
Dipeptides	
(Phe-Ala, Gly-Gly, Gly-Pro, Phe-Pro, Ile-Ala, Ala-Pro, Ala-Ala, Tyr-Pro)	no effect
Inhibitors of DP IV enzymatic activity	
A-Ala-Pro-O(nitrobenzoyl-)hydroxylamine, Lys[Z(NO_2)]-thiazolidide, Lys[Z(NO_2)]-piperidide)	no effect
Cytokines and growth factors	
(human IL-2, murine IL-2, human IL-1b, human ACTH)	no effect
ECM proteins	
human fibronectin	18.9 ± 2.9
collagen	no effect
Protein mixtures	
(gelatine, peptone, casiton)	no effect
Albumins	
(human, bovine)	no effect
Basic proteins	
(ribonuclease, protamin)	no effect
Acidic proteins	
(amyloglucosidase)	no effect
Lectins	
AAA *(Aleuria aurantia agglutinine)*	9.8 ± 3.8
MAA *(Maackia amurensis agglutinine)*	56.2 ± 8.7
WGA (*Triticum vulgaris agglutinine)*	90.2 ± 5.2
RCA *(Ricinus communis agglutinine*)	89.4 ± 3.6
SNA (*Sambucus niger agglutinine)*	79.7 ± 2.1
PHA (*Phaseolus vulgaris agglutinine)*	10.6 ± 8.2
PWM (*Phytolacca americana agglutinine)*	4.7 ± 7.5

[1]DP IV-ADA interactions were analyzed using a dot-blot assay (n = 3) based on interaction of biotinylated ADA with purified human DP IV immobilized onto nitrocellulose as described [8]. The concentration of the putative competitors has been titrated to saturation of the effect. Reagents were obtained from Boehringer Ingelheim, Sigma and from sources as described elsewhere [8].

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