Signaling via dopamine and adenosine receptors modulate viral peptide-specific and T-cell IL-8 response in COVID-19

Mieko Tokanōa,b, Rie Takagia, Masaaki Kawanōa, Shigefumi Maesakib, Norihito Tarumotob and Sho Matsushitaa,c

aDepartments of Allergy and Immunology, Faculty of Medicine, Saitama Medical University, Moroyama, Saitama, Japan; bDepartment of Infectious Disease and Infection Control, Saitama Medical University, Moroyama, Saitama, Japan; cAllergy Center, Saitama Medical University, Moroyama, Saitama, Japan

ABSTRACT

B-cell but not T-cell responses have been extensively studied using peripheral blood mononuclear cells (PBMCs) obtained from patients with coronavirus disease 2019 (COVID-19). Our recent study showed that not only T-helper (Th) 17 but also Th1 cells directly produce interleukin (IL)-8, a major source of neutrophilic inflammation, which is also known to induce disseminated intravascular coagulation (DIC) in COVID-19 patients. Neutrophilic inflammation caused by IL-17A or IL-8 can be fatal; thus, therapeutic intervention is highly expected. The present study aimed to investigate the T-cell responses in the Japanese patients. We synthesized spike protein-derived 15-mer peptides that are expected to bind to HLA class II allelic products frequently observed in the Japanese population, and checked the T-cell responses in Japanese patients with COVID-19. We have found that (i) patients show marked IL-8 but not IL-17A responses; (ii) these responses are restricted by HLA-DR; and (iii) IL-8 responses are abrogated by a dopamine D2 like receptor (D2R) agonist, ropinirole, and an adenosine A2a receptor (A2aR) antagonist, istradefylline. Compounds used for the treatment of Parkinson’s disease may ease DIC in COVID-19. (183 words)

1. Introduction

Corticosteroids are effective for easing eosinophilic but not neutrophilic inflammation; the latter includes the wide spectrum of diseases, such as various autoimmune diseases [1–4], neutrophilic airway inflammation [5,6] and disseminated intravascular coagulation (DIC) in coronavirus disease 2019 (COVID-19) [7]. COVID-19 is a disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Peripheral blood mononuclear cells (PBMCs) obtained from patients with COVID-19 reportedly show peptide-specific interleukin (IL)-17 and IL-8 responses [8], yet there have been no reports using Japanese patients. Neutrophilic inflammation caused by IL-17A or IL-8 is known to induce DIC via neutrophil extracellular traps (NETs) [7], which together with the cytokine storm may be fatal; thus, therapeutic intervention is highly expected.

With regard to therapeutic intervention, we have shown that dopamine D2 receptor (D2R) agonists and an adenosine A2a receptor (A2aR) antagonist are capable of inhibiting not only IL-17A responses in various animal models in vivo [1–6], but also IL-8 responses to natural antigens in humans [9,10]. One of these animal models includes a rheumatoid arthritis-severe combined immunodeficiency mouse model with human synovial tissue [4], which attests to the effectiveness in humans. Mechanisms for the effect of adenosine on T-cell responses are schematically outlined in Figure 1. The major source of adenosine is CD4+ T cells, whereas that of dopamine is dendritic cells [1–6]. In the present study, we selected and synthesized nine 15-mer peptides derived from SARS-CoV-2 spike protein, which are expected to bind to human leukocyte antigen (HLA) class II allelic products frequently observed in the Japanese population, using a reverse immunogenetic analysis that was performed by other researchers [11]. PBMCs from twenty-three Japanese patients with COVID-19 were co-incubated with these peptides to investigate cytokine profiles.

2. Materials and methods

2.1. Participants

The present study included 23 Japanese patients with laboratory-confirmed COVID-19 who were admitted to Saitama Medical University Hospital in

CONTACT Sho Matsushita shomat@saitama-med.ac.jp Department of Allergy and Immunology, Faculty of Medicine, Saitama Medical University, 38 Morohongo, Moroyama, 350-0495, Saitama, Japan

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Informed consent was obtained from all study participants. Laboratory-confirmed COVID-19 was defined by the detection of SARS-CoV-2 RNA by reverse transcription-polymerase chain reaction using a nasopharyngeal swab and sputum sample. We treated the patients according to the COVID-19 clinical guidelines issued by the Ministry of Health, Labor and Welfare, Japan [12]. Patients who met the criteria for releasing quarantine provided 20 mL peripheral venous blood. These samples were subjected to the isolation of PBMCs.

2.2. Preparation of PBMCs

Peripheral blood was obtained from the patients under protocols approved by the Institutional Review Board (No. 2021-063) of Saitama Medical University Hospital. The blood was centrifuged for 10 min at 450 \( \times \) g, and separated into blood cells and plasma. After adding RPMI1640 (Sigma-Aldrich, St. Louis, MO, USA) to the blood cells, the sample was overlaid onto a Ficoll-Paque PLUS (GE Healthcare, Buckinghamshire, England, UK) and centrifuged for another 40 min at 450 \( \times \) g. PBMCs were recovered from the top-most layer of Ficoll-Paque.

2.3. Peptides and monoclonal antibodies

Nine 15-mer peptides derived from the spike protein of SARS-CoV-2, as shown in Table 1, were synthesized in Eurofins (Tokyo, Japan). These peptides do not contain any point mutation sites observed in alpha, beta, gamma, or delta strains. PBMCs (1.5 \( \times \) 10^5 cells/well in a 96-well flat-bottomed culture plate) at 37°C were stimulated with peptides (5 \( \mu \)M) in 100 \( \mu \)L of RPMI 1640 (Sigma-Aldrich) medium containing 10% human serum, 1% L-glutamine, 50 IU/mL penicillin, and 50 \( \mu \)g/mL streptomycin (R10H medium) to characterize the cytokine production patterns. PBMCs obtained from patients C-04, C-11, and C-13 were incubated with or without 10 \( \mu \)g/mL mouse monoclonal antibodies (Abs) (L243, HU-4, 1a3), istradefylline (10 \( \mu \)g/mL), and ropinirole (10 \( \mu \)g/mL), and Cmax; Maximum Plasma Concentration, where Cmax is 170 ng/mL or ropinirole (10 \( \mu \)g/mL, and 1 \( \mu \)g/mL; Maximum Plasma Concentration, where Cmax is 1.5 ng/mL) in 100 \( \mu \)L of R10H medium. Anti-HLA class II monoclonal Abs HU-4 (anti-HLA-DRB1 \( ^{+} \)B5 IgG2a, monomorphic) and L243 (anti-HLA-DRB1 \( ^{+} \)DRB3 \( ^{+} \)DRB4 IgG2a, monomorphic) were as described [13]. Anti-HLA class II monoclonal Ab 1a3 (anti-HLA-DQ IgG2a, monomorphic) (Leinco Technologies, Manchester, U.K.) was purchased [13]. At seven days after the initiation of the culture, the supernatants were collected for cytokine ELISAs.

2.4. Cytokine ELISAs

The concentrations of IL-5, IL-8, IL-17A, and interferon (IFN) \( \gamma \) in culture supernatant fluids were measured using specific ELISA kits (DuoSet Kit, R&D). Any value below the lower limit of detection (15.6 pg/mL) was set to 0. No cytokine cross-reactivity was observed within the detection ranges of the kits. If necessary, samples were diluted appropriately so that the measurements fell within the appropriate detection range for each cytokine.

2.5. Statistical analysis

Differences between three or more groups were analyzed using a one-way ANOVA with Tukey’s post-hoc test. The correlation between IL-8 and clinical parameters was analyzed using Spearman’s ranking correction. The relationship between IL-8 and oxygen inhalation was analyzed using a non-parametric Mann-Whitney U-test. All calculations were performed using the KaleidaGraph software program.

Table 1. The 15-mer peptides derived from the spike protein of SARS-CoV-2.

| Peptide | Amino acid sequence | Position | Expected restriction |
|---------|---------------------|----------|----------------------|
| A       | CTFEYVQFPMLMDLE    | P166-180 | DQ                   |
| B       | NIDQTKY05KHTPI      | P196-210 | DR                   |
| C       | LMDFGKQGKFNLR       | P176-190 | Class II             |
| D       | CEFQNDPFLGYY       | P131-145 | DQ                   |
| E       | SSANNCTFEYQQSPF     | P161-175 | Class II             |
| F       | RFASYAVWNRKKSN      | P346-360 | DR                   |
| G       | AGPKOYDCGLDA        | P831-845 | DQ                   |
| H       | EFVKNIDGITYOSY      | P191-205 | DQ                   |
| I       | LPQGSFALPLVDLP      | P216-230 | DQ                   |
(Synergy software, Reading, PA, USA). p Values of <.05 were considered to be statistically significant.

3. Results

The cytokine profiles of all patients are shown in Table 2. Data are expressed as the mean of triplicate determinations. All patients, with the exception of C-17, showed a marked IL-8 response, whereas no patients showed marked IL-5/IL-17A responses (not shown). C-15 alone showed an IFNγ response (1.6 ng/mL, not shown). The largest IL-8 responses in all patients were converted into rank variables, and the statistical associations with clinical parameters were analyzed. IL-8 values were significantly associated with CRP (p = .03), whereas the associations with oxygen inhalation and the neutrophil counts were marginal (p = .06 and .07, respectively). The IL-8 values without peptides vary among the patients, between 0.9 and 10 ng/mL. However, the levels showed statistical association neither between CRP, oxygen inhalation, nor neutrophil counts (p ≥.11). We also checked three healthy individuals after vaccination without COVID-19 history. They showed less than 1.5 ng/mL of IL-8 values in peptide-specific manners (not shown).

We then selected 3 patients whose frozen PBMCs were sufficiently obtained for the following experiments, by which HLA restriction patterns were determined. As shown in Figure 2, anti-HLA-DR Abs HU-4 and L243, but not anti-DQ Ab 1a3, significantly inhibited the IL-8 responses in all 3 patients.

We then set up an experiment simultaneously to test the effect of a D2R agonist, ropinirole, and an A2aR antagonist, instradefyllin, on IL-8 responses in the 3 patients. As shown in Figure 3, all peptide-specific IL-8 responses were inhibited by these compounds, at all of the tested concentrations, where the concentration (1 × Cmax) is physiologically feasible.

4. Discussion

Anti-HLA-DR Abs HU-4 and L243, but not anti-DQ Ab 1a3, significantly inhibited the IL-8 responses in all 3 patients, indicating that: 1) peptides are presented by the HLA class II molecules (HLA-DR); and 2) cluster of differentiation (CD)4+ T cells produce IL-8. In our previous studies, we showed that D1R antagonists/D2R agonists [1–4,6] and an A2aR antagonist [5,10,14] suppressed IL-17A but not IL-5 responses, in both mice and humans. High level of the IL-8 production in humans was observed in human T-helper (Th)1 and Th17 cells, but not Th2 cells in our previous study [9].

Table 2. Cytokine profiles of all patients.

| Pt. ID | ΔIL-8 |
|-------|-------|
| C-01  | 0.1D  |
| C-02  | 26.5F, 18.8C |
| C-03  | 2.2A  |
| C-04  | 0.5F  |
| C-05  | 1.6L, 0.8H, 0.1G |
| C-06  | 3.4C  |
| C-07  | 0.7B  |
| C-08  | 2.4D  |
| C-09  | 1.8H  |
| C-10  | 1.0F  |
| C-11  | 8.2F, 8.2B |
| C-12  | 0.6L, 0.5F |
| C-13  | 17.1A |
| C-14  | 1.1H  |
| C-15  | 28.3A |
| C-16  | 0.8F  |
| C-17  | (−)   |
| C-18  | 0.4F, 0.4H |
| C-19  | 2.6F  |
| C-20  | 2.2C, 2.1F |
| C-21  | 2.6F  |
| C-22  | 3.6C, 3.5F |
| C-23  | 4.7F  |

ΔIL-8 was defined as ‘(IL-8 production incubated with PBMCs plus a peptide) — (IL-8 production incubated with PBMCs only)’. The letters after the numbers indicate the type of peptide. For example, 26.5F means 26.5 ng/mL of IL-8 was induced with peptide F. (−): <0.1 ng/mL.

Figure 2. PBMCs obtained from three patients were incubated in the presence of peptides with or without mouse monoclonal Abs. After 7 days, culture supernatant fluids were collected for ELISAs (n = 2). Data are expressed as the mean ± standard deviation (SD) and were compared using a one-way ANOVA with Tukey’s post-hoc test. **p < 0.01, in comparison to medium plus peptide.
All peptide-specific IL-8 responses were inhibited by ropinirole and istradefyllin, at all of the tested concentrations. As a control, we observed the granulocyte macrophage colony-stimulating factor responses (not shown), but they were not inhibited, indicating that the effects are IL-8-specific. The evaluation of Th2 responses in the human 7-day-culture can be well achieved using IL-5 but not IL-4, due to their kinetics [13], while human T cells that produce IL-5 but not IL-4 have never been reported. Because patients can produce neutralizing Abs to the spike protein, it would be interesting to know whether they show T-follicular-helper-cell responses in their lymph nodes; however, such an experiment would be ethically unfeasible.

DIC induced by NETs formation is an important cause of acute respiratory distress syndrome (ARDS) in patients with COVID-19. Indeed, many patients showed marked peptide-specific IL-8 responses alone in this study. Studies by others demonstrated that IL-8 produced under Th17 polarizing conditions in the presence of aryl hydrocarbon receptor (AHR) activation is up-regulated [15]. Indeed, certain viral infections, such as coronaviruses and Zika virus reportedly induce kynurenine production, which activates AHR [16]. Both istradefylline and ropinirole are commercially available to treat Parkinson’s disease. They are capable of suppressing IL-8/17 responses but not Th2, suggesting that they should inhibit harmful neutrophilic inflammation without downregulating the production of neutralizing Abs. In this relation, numerous studies have reported a reduced risk of rheumatoid arthritis (a typical IL-17A-associated disease) in schizophrenia [17], carrying characteristics opposite to Parkinson’s disease, i.e. excessive stimulation via D2-like receptors [18]. It is thereby feasible that Parkinson’s disease medication may ease T-cell-mediated neutrophilic inflammation and associated DIC.

IL-17A is known to affect both chemotaxis and differentiation of neutrophils. Moreover, the compounds shown in this study not only suppress IL-17A responses but also affect other neutrophil-associated cytokines such as IL-17F and IL-22 [14]. Related compounds also affect natural immunity shown in our previous study [1,2]. Inhibitory spectrum of istradefylline and ropinirole on neutrophilic inflammation is thereby expected to be wider than IL-8 inhibition alone, and is currently under investigation into details. In addition, eosinophilic inflammation caused by type I allergy is usually accompanied by neutrophilic inflammation, especially in severe atopic dermatitis and corticosteroid-resistant bronchial asthma frequently observed in elders [6]. Combination of corticosteroids and istradefylline/ropinirole may ease such pathology.

Although the source of information is limited to three patients, the antigen-presenting molecule was HLA-DR, even in the case of the predicted presentation by HLA-DQ (peptide A as shown in Table 1). It is conceivable that the structural motifs for the peptides that bind to HLA-DR and DQ have much in common [19,20]. From a functional point of view, signals are transmitted to antigen presenting cells (APCs) via HLA molecules in antigen presentation [13]. Studies by ourselves and others showed that antigen presentation by DR and DQ molecules can lead to the differential activation of mitogen-activated protein kinase (MAPK) and monokines [13], and that Th1 and Th2 cells tend to be activated by the presentation of DR and DQ molecules, respectively [13,21]. Currently, 5 subfamilies have been reported in MAPK, and it would be interesting to know which subfamilies are activated after the
presentation by DR or DQ, which might be associated with the activation of IL-8 produced by T cells. Such a study is currently underway. HLA is highly polymorphic, and the frequency of each allele varies in various ethnic groups, suggesting that different SARS-CoV-2 spike protein fragments can be presented in different ethnic groups. HLA class I-restricted responses of CD8\(^+\) T cells are also under investigation.

**Ethical approval**

The protocol for this research project was approved by the Institutional Review Board (No. 2021-063) of Saitama Medical University Hospital, and conforms to the provision of Declaration of Helsinki. Informed consent was obtained from all participants.

**Author contributions**

M.T., R.T., and S.Mat., performed the experiments. M.T., N.T., and S.Mae treated the patient. M.T. and N.T. collected the data. M.T., R.T., M.K. and S.Mat., conceived and designed the experiments. M.T. and S.Mat., wrote the manuscript. All authors discussed the results and commented on the manuscript.

**Disclosure statement**

Sho Matsushita is an employee of iMmno, Inc. No potential conflict of interest was reported by the author(s).

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**ORCID**

Sho Matsushita [http://orcid.org/0000-0003-4810-1065](http://orcid.org/0000-0003-4810-1065)

**References**

[1] Kawano M, Takagi R, Kaneko A, et al. Berberine is a dopamine D1- and D2-like receptor antagonist and ameliorates experimentally induced colitis by suppressing innate and adaptive immune responses. J Neuroimmunol. 2015;289:43–53.

[2] Kawano M, Takagi R, Saika K, et al. Dopamine regulates cytokine secretion during innate and adaptive immune responses. Int Immunol. 2018;30(12):591–606.

[3] Kawano M, Saika K, Takagi R, et al. Tannic acid acts as an agonist of the dopamine D2L receptor, regulates immune responses, and ameliorates experimentally induced colitis in mice. Brain Behav Immun Health. 2020;5:100071.

[4] Nakano K, Yamaoka K, Hanami K, et al. Dopamine induces IL-6-dependent IL-17 production via D1-like receptor on CD4 naïve T cells and D1-like receptor antagonist SCH-23390 inhibits cartilage destruction in a human rheumatoid arthritis/SCID mouse chimera model. J Immunol. 2011;186(6):3745–3752.

[5] Tokano M, Kawano M, Takagi R, et al. Istradefylline, an adenosine A2a receptor antagonist, ameliorates neutrophilic airway inflammation and psoriasis in mice. Clin Exp Neuroim. 2021;12(4):268–275.

[6] Nakagome K, Imamura M, Okada H, et al. Dopamine D1-like receptor antagonist attenuates Th17-mediated immune response and ovalbumin antigen-induced neutrophilic airway inflammation. J Immunol. 2011;186(10):5975–5982.

[7] Middleton EA, He X, Denorme F, et al. Neutrophil extracellular traps contribute to immunothrombosis in COVID-19 acute respiratory distress syndrome. Blood. 2020;136(10):1169–1179.

[8] Karolina J, Jablonska E, Garley M. Significance of NETs formation in COVID-19. Cells. 2021;10(1):151.

[9] Matsuyama T, Kawano M, Takagi R, et al. IL-8 produced by T cells is under the control of dopamine signaling. Clin Exp Neuroimunol. 2018;9(4):251–257.

[10] Tokano M, Kawano M, Takagi R, et al. Istradefylline, an adenosine A2a receptor antagonist, inhibits the CD4+ T-cell hypersecretion of IL-17A and IL-8 in humans. Immunol. Med. in press.

[11] Alba G, Sidney J, Vita R, et al. SARS-CoV-2 human T cell epitopes: adaptive immune response against COVID-19. Cell Host Microbe. 2021;29(7):1076–1092.

[12] Adachi T, Ayusawa M, Ujije M, et al. Novel coronavirus infection COVID-19 medical practice guideline, version 6.1; 2022 [accessed 2022 Jan 10]. Available from: https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/0000121431_00111.html.

[13] Matsuoka T, Tabata H, Matsushita S. Monocytes are differentially activated through HLA-DR, -DQ, and -DP molecules via mitogen-activated protein kinases. J Immunol. 2001;166(4):2202–2208.

[14] Tokano M, Matsushita S, Takagi R, et al. Extracellular adenosine induces hypersecretion of IL-17A by T-helper 17 cells through the adenosine A2a receptor to promote neutrophilic inflammation. bioRxiv. 2021:441713.

[15] Michaela G, Bauer M, Hinz D, et al. Generation of intraindividual and familial risks. Schizophr Bull. 2014;40:1552–1559.

[16] Abi-Dargham A, Rodenhisler J, Printz D, et al. Increased baseline occupancy of D2 receptors by...
[19] Matsushita S, Takahashi K, Motoki M, et al. Allele specificity of structural requirement for peptides bound to HLA-DRB1*0405 and -DRB1*0406 complexes: Implication for the HLA-associated susceptibility to methimazole-induced insulin autoimmune syndrome. J Exp Med. 1994;180(3): 873–883.

[20] Oiso M, Nishi T, Ishikawa T, et al. Differential binding of peptides substituted at putative C-terminal anchor residues to HLA-DQ8 and DQ9 differing only at beta 57. Hum Immunol. 1997;52(1): 47–53.

[21] Chapoval SP, Nabozny GH, Marietta HV, et al. Short ragweed allergen induces eosinophilic lung disease in HLA-DQ transgenic mice. J Clin Invest. 1999;103(12):1707–1717.