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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Antiviral response in vernal keratoconjunctivitis may be protective against COVID-19

To the Editor,

Vernal keratoconjunctivitis (VKC) is a severe type 2 ocular eosinophilic inflammation with a proven IgE sensitization in about 50% of patients. Many Th2-type and proinflammatory cytokines have been found to be locally overexpressed in VKC patients, recalling a sort of local cytokine storm. Conjunctivitis is a common, self-limiting manifestation of COVID-19 with an incidence of 11% in affected patients,[1] but can be the first or the unique manifestation of SARS-CoV-2 infection. As a referral center for the diagnosis and treatment of VKC, so far, we observed only two VKC patient affected by COVID-19 without any ocular symptoms or consequences. The prevalence of VKC is estimated in our area 4/10.000 under 15 years of age.[2] Knowing that the prevalence of COVID-19 in pediatric population (0–14) in Padova great area is 6.4%, we calculated that the odds ratio (OR) for VKC to be associated with COVID-19 is OR = 0.88 (95% CI, 0.66–1.16), therefore, with a tendency for VKC to be protective. It has been suggested that a Th2-skewed immunity may be protective against severe COVID-19 disease.[3] For this reason, we investigated the conjunctival expression of genes related to the local defense immunity to virus that may play a relevant role in the response to SARS-CoV-2.

Conjunctival samples were collected from 15 VKC patients and 5 healthy age-matched control subjects (CTRL) using the Eyeprim™ device (OPIA Technologies SAS). Samples were immediately treated and stored at −80°C for subsequent RNA isolation and Affymetrix assay (see Appendix S1). Over the 21,448 tested expression probes, using the Gene Ontology Biological Process (GOBP) term "defense response to virus," 237 genes were selected (Figure S1). In addition, using bibliographic elements, we selected genes with SARS-CoV-2 receptor function and antiviral activity. The receptor angiotensin-converting enzyme 2 (ACE2), cellular transmembrane serine protease 2 (TMPRSS2), Basigin/CD147/EMMPRIN (BSG), cathepsin L (CTSL), and dipeptidyl peptidase (DPP4) were not overexpressed in VKC compared to CTRL. Conversely, FURIN (FC = 2.73, $p = 0.001$) and ADAM-17 (FC = 1.61; $p = 0.01$) were significantly higher in VKC. Thirty-eight genes involved in the defense response to virus, including bone marrow stromal antigen (BST2)/tetherin and MX Dynamin Like GTPase 2/myxovirus resistance protein 2 (MX2) and tumor necrosis factor-alpha-induced protein 3 (TNFAIP3) were overexpressed in VKC (Table 1 and Figure 1). Even though several members of the interferon regulatory and inducible proteins (Table 1) were overexpressed in VKC, genes encoding for interferons were not. Notably, interferon receptors IFNAR1 (FC = 1.88; $p = 0.003$), IFNGR2 (FC = 2.6; $p = 0.04$) were significantly overexpressed in VKC.

The meaning of the upregulation of all these antiviral genes in VKC is not clear. It has been shown that ACE2 is overexpressed in diseased conjunctiva compared to normal tissues and that conjunctival inflammation can enhance its expression.[4] Our results show that this is not the case for allergic inflammation. It has been suggested that type 2 immune response can provide certain protective effects against COVID-19 since asthma patients do not have

TABLE 1 Significantly overexpressed antiviral genes in VKC compared with control (CTRL)

Transcript cluster id	Symbol	Description	VKC vs CTRL FC	*p* value
TC0100015921.hg.1	ADAR	Adenosine deaminase, RNA-specific	2.62	.0016
TC1100006500.hg.1	AP2A2	Adaptor-related protein complex 2, alpha 2 subunit	2.79	.0000
TC2200009268.hg.1	APOBEC3A	Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3A	1.42	.0045
TC2200009271.hg.1	APOBEC3C	Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3C	2.19	.0069
TC1900009859.hg.1	BRD4	Bromodomain containing 4	2.78	.0061
TC1900009970.hg.1	BST2	Bone marrow stromal cell antigen 2	11.59	.0016
TC0500012470.hg.1	CD74	CD74 molecule, major histocompatibility complex, class II invariant chain	4.03	.0107
TC1700010698.hg.1	CNP	Memczak2013 ANTISENSE, CDS, coding, INTERNAL best transcript NM_033133	1.77	.0052
TC1100006840.hg.1	CTR9	CTR9 homolog, Paf1	1.41	.0534
TC0200012257.hg.1	EIF2AK2	Eukaryotic translation initiation factor 2-alpha kinase 2	2.74	.0028
TC1100012949.hg.1	IFITM1	Interferon induced transmembrane protein 1	3.33	.0417
TC0500012017.hg.1	IRF1	Interferon regulatory factor 1	2.83	.0382
TC1600008712.hg.1	IRF8	Interferon regulatory factor 8	1.65	.0456
TC1400010584.hg.1	IRF9	Interferon regulatory factor 9	1.52	.0442
TC0100010244.hg.1	IFI16	Interferon, gamma-inducible protein 16	2.83	.0005
TC0200014772.hg.1	IFIH1	Interferon induced, with helicase C domain 1	4.64	.0009
TC0100006483.hg.1	ISG15	ISG15 ubiquitin-like modifier	5.91	.0371
TC1700011922.hg.1	LGALS3BP	Lectin, galactoside-binding, soluble, 3 binding protein	3.07	.0018
TC0100009449.hg.1	MOV10	Mov10 RISC complex RNA helicase	2.16	.0027
TC2100007205.hg.1	MX2	MX dynamin-like GTPase 2	14.65	.0027
TC2000009023.hg.1	SAMHD1	SAM domain and HD domain 1	3.20	.0014
TC1200010908.hg.1	STAT2	Signal transducer and activator of transcription 2	3.69	.0001
TC0600009597.hg.1	TNFAIP3	Tumor necrosis factor, alpha-induced protein 3	9.75	.0015
TC0800007007.hg.1	TNFRSF10C	Tumor necrosis factor receptor superfamily, member 10c, decoy without an intracellular domain	2.50	.0415
TC1100009940.hg.1	TRIM5	Tripartite motif containing 5	1.66	.0231
TC0100017612.hg.1	TRIM11	Tripartite motif containing 11	1.56	.0079
TC0900010968.hg.1	TRIM14	Tripartite motif containing 14	1.83	.0151
TC1700007135.hg.1	TRIM16L	Tripartite motif containing 16-like	2.13	.0246
TC1100009942.hg.1	TRIM22	Memczak2013 ANTISENSE, CDS, coding, INTERNAL, UTR5 best transcript NM_001199573	2.44	.0007
TC1100012957.hg.1	TRIM22	Tripartite motif containing 22	2.25	.0189
TC1700011208.hg.1	TRIM25	Tripartite motif containing 25	2.08	.0040
TC0600011351.hg.1	TRIM31	Tripartite motif containing 31	4.89	.0504
TC0100015346.hg.1	TRIM33	Tripartite motif containing 33	1.41	.0385
TC0600007257.hg.1	TRIM38	Tripartite motif containing 38	2.06	.0477
TC0500009762.hg.1	TRIM41	tripartite motif containing 41	1.85	.0434
TC0700008579.hg.1	TRIM56	tripartite motif containing 56	2.47	.0397
TC0100012328.hg.1	TRIM58	tripartite motif containing 58	2.58	.0037
TC0100007832.hg.1	ZC3H12A	Zinc finger CCCH-type containing 12A	3.71	.0024

Abbreviations: CTR, controls; FC, fold change; VKC, vernal keratoconjunctivitis.

increased susceptibility or severity of SARS-CoV-2 infection than others.[5] Although the presence of SARS-CoV-2 in tears has rarely been detected in infected individuals, the conjunctiva is a potential gateway for the SARS-CoV-2 and conjunctivitis may be a sign of COVID-19 prior to or after the onset of respiratory symptoms.

In our study, we suggest that the overexpression of multiple antiviral factors in severe allergic inflammation and the low ACE2 expression in the conjunctiva might explain to the low prevalence of COVID-19 in VKC. In addition, it has been shown that having eosinophilia and a Th2 phenotype (which are typical of VKC) may

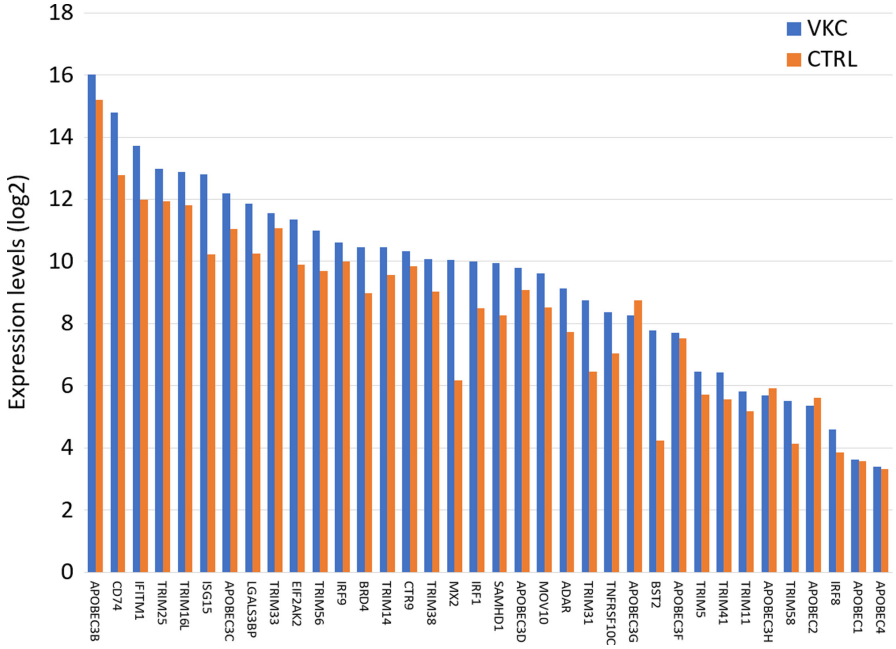

FIGURE 1 Histogram showing the levels of expression of selected genes in VKC patients and control subjects (CTRL). The selection includes genes involved in the antiviral response. VKC, vernal keratoconjunctivitis

be an important predictive factor for reduced COVID-19 morbidity and mortality in asthma.[6] We cannot translate this statement for VKC patients, but we suggest that having a local persistent allergic inflammation might be protective for ocular viral infections.

KEYWORDS

ACE2, antiviral factors, COVID-19, SARS-CoV-2, vernal keratoconjuntivitis

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CONFLICT OF INTEREST

Authors have no conflict of interest, only Philippe Daull and Jean-Sébastien Garrigue are employees of Santen SAS.

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