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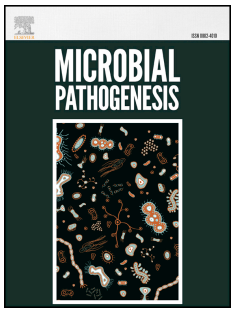

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Altered expression of cytokines in mice infected intranasally with two syncytial variants of Herpes simplex virus type 1

Keywords: herpes, virulence, cytokines, pathology, mouse

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Abstract

Immune evasion strategies are important for the onset and the maintenance of viral infections. Many viruses have evolved mechanisms to counteract or suppress the host immune response. We have previously characterized two syncytial (syn) variants of Herpes simplex 1 (HSV-1) strain F, syn14-1 and syn17-2, obtained by selective pressure with a natural carrageenan. These variants showed a differential pathology in vaginal and respiratory mucosa infection in comparison with parental strain. In this paper we evaluated the modulation of immune response in respiratory mucosa by these HSV-1 variants. We observed altered levels of Tumor Necrosis Factor-α and Interleukin-6 in lungs of animals infected with the syn14-1 and syn17-2 variants compared with the parental strain. Also, we detected differences in the recruitment of immune cells to the lung in syn variants infected mice. Both variants exhibit one point mutation in the sequence of the gene of glycoprotein D detected in the ectodomain of syn14-1 and the cytoplasmic tail of syn17-2. Results obtained in the present study contribute to the characterization of HSV-1 syn variants and the participation of the cellular inflammatory response in viral pathogenesis.

Main text

Herpes simplex virus type 1 (HSV-1) is a human pathogen that infects and replicates in epithelial cells of mucosal surfaces with the eventual establishment of latency in the ganglia of sensory neurons. Important roles in limiting spread and early virus replication have been ascribed to macrophages and natural killer cells (NK), which constitute the first line of innate defense [1-3]. As expected, HSV-1 has evolved several strategies to counteract the antiviral response by preventing the infected host to trigger a strong immune response and thus facilitating viral replication. Furthermore, it has been previously reported that HSV is able to suppress expression of proinflammatory cytokines by decreasing the stability of mRNAs [4]. Secretion of proinflammatory cytokines is essential in the first line defense against viruses [5, 6]. One of the macrophages-derived products that contribute to inhibit HSV replication is the proinflammatory cytokine TNF-α [7] that plays an important role on the early innate immune response. Furthermore, macrophages and other cell types rapidly produce IL-6 at local tissue sites after HSV-1 infection [8]. IL-6 is a cytokine with pleiotropic activities, including both proinflammatory and anti-inflammatory effects, that has been characterized to contribute to immune response to HSV-1. At the same time, anti-inflammatory cytokines are immunoregulatory molecules that control the proinflammatory cytokine response to impair an excessive response [9, 10].

Natural carrageenans are known to be potent and selective inhibitors of HSV-1 and HSV-2, affecting mainly the viral adsorption step [11]. As a result of a previous work we performed we found that the selective pressure with the μ/ν- carrageenan 1C3 allowed the isolation of two syncytial (syn) variants of HSV-1 F strain, syn14-1 and syn17-2 which showed an altered pathology in vaginal and respiratory mucosa infection [12]. In fact, intranasal infection of BALB/c mice with syn variants induced 100% mortality at 7 days post-infection (p.i.) and a differential infiltration of leukocytes was observed by histopathological studies from lung mucosa.

Although HSV-1 is not a common respiratory virus in human, it can cause several pathological conditions associated with the respiratory tract. In fact, herpetic respiratory infections have been reported not only in neonates and immunosuppressed patients [13, 14], but also in immunocompetent ones [15, 16]. Moreover, HSV-1 is able to penetrate the

basement membrane of human nasal respiratory mucosa and to replicate both in epithelium and the underlying lamina propria of this tissue [17].

Based on these data, in the present work we wanted to deepen the study of the intranasal infection of mice with variants syn14-1 and syn17-2 towards a better understanding of the participation of the cellular inflammatory response in the pathogenicity of these viruses. Taken into account the relevance of a rapid secretion of cytokines to counteract viral infections, we focused on the analysis of cytokine modulation in lungs of infected mice. To this end, BALB/c mice were infected with 10^6 PFU of syn14-1, syn17-2 or HSV-1 F strain, as a control. At day 1 and 3 p.i., bronchioalveolar lavages (BAL) were performed using 1 ml of sterile saline solution. TNF-α, IL-6 and IL-10 levels were studied by ELISA according to manufacturer´s instructions (BD Biosciences) and virus yield was quantified by plaque assay. Animals were maintained and handled in accordance with national and international laws and policies from National Institutes of Health Guidelines and regulations for care and use of test animals from Facultad de Ciencias Exactas y Naturales (Buenos Aires, Argentina, CD 140/00).

Results shown in Fig. 1a (white bars) demonstrate that reduced levels of TNF-α could be detected at day 1 p.i. for syn14-1 and syn 17-2. This low values were similar to those observed in uninfected animals (1023,48 pg/ml). In contrast , at day 3 p.i. the level of TNF-α for both syn variants was increased, while the parental strain showed a diminution on TNF-α production (Figure 1a, grey bars). When IL-6 levels were analyzed, we found that day 1 p.i. syn14-1 values showed an increase in IL-6 production while syn17-2 showed a marked reduction in comparison with HSV-1 F, resembling the value of negative control (28,31 pg/ml) (Figure 1b, white bars). However, at day 3 p.i. no significant differences were observed for IL-6 levels in infected mice either with HSV-1 F or syn variants (Figure 1b, grey bars). The differential levels in cytokine production observed for syn variants could be due to an over-expression of inhibitory cytokines. To test this possibility, our next approach was to evaluate the production of IL-10, one of the major antiinflammatory cytokines. As shown in Figure 1c, levels of IL-10 for syn14-1 and syn17-2 were lower at 1 day p.i. (white bars) similar of control without infected (1157,53 pg/ml), and increased at 3 days p.i. (grey bars) in comparison with parental virus. According to these results, the pattern of IL-10 production observed for HSV-1 F and both syn variants was coincident

with the course of induction of TNF-α, suggesting that reduction of TNF-α observed 1 day p.i was not due to an overproduction of IL-10. The proinflammatory cytokine IL-6 would be also regulated by IL-10 [9], however the induction of this cytokine inhibitor would not seem to explain completely the level of IL-6 observed at least for syn 14-1 variant.

With the aim to evaluate the correlation of cytokines pattern with virus replication, virus titer was quantified in lung of infected mice. As can be seen in Figure 1d, at day 1 p.i., viral titers for syn14-1 and syn17-2 were 10 and 113-fold higher, respectively, than those registered for mice infected with HSV-1 F. At day 3 p.i., virus titers for syn variants as well as for the parental strain were negligible.

These results suggest that intranasally inoculated syn variants would be able to modulate differentially the immune response in association with a higher replication in lungs of infected mice. At the same time, this modulation could explain the high mortality previously described for mice intranasally infected with syn14-1 and syn17-2 [12]. Therefore, it may be speculated that the increased replication of syn variants might contribute to their pathogenic phenotypes.

Considering that alveolar macrophages constitute the main cell population present in the BAL fluid, we decided to analyze whether infection with HSV-1 F and syn variants affected cytokine release in an *in vitro* model employing the murine macrophagic cell line RAW 264.7. For this purpose, cells were infected with HSV-1 F, syn14-1 and syn17-2 and at different times p.i., supernatants and cells monolayers were harvested and TNF-α and IL-6 were determined by ELISA and RT-PCR, respectively. Cells stimulated with LPS (1.5µg/ml) were used as positive control. For PCR analysis, cell monolayers were lysed in Trizol (Invitrogen) and total RNA was isolated according to manufacturer's instructions. cDNA was amplified with an initial incubation at 94°C during 10 min followed by 35 cycles of 1 min at 94°C, 1 min at 60°C and 1 min at 72°C and a final incubation of 10 min at 72°C. β-actin was used as an internal control. As can be seen in Fig. 2a, the levels of the proinflammatory cytokines TNF-α and IL-6 were markedly reduced in cells infected with syn14-1 and syn17-2, in contrast to those observed for HSV-1 F. In fact, the concentration of both cytokines diminished by 84 to100 % for the syn variants with respect to HSV-1 F for every tested time. Furthermore, results obtained by RT-PCR correlated with those obtained by ELISA since low levels of IL-6 and TNF-α mRNAs were also detected for the

syn variants (Fig. 2b) indicating that the transcription of these cytokines genes would not be affected. Similar results were obtained using intraperitoneal macrophages harvested from BALB/c mice (data not shown). The lower levels of cytokines observed by day 1 p.i. in lung of mice infected intranasally with the syn 17-2 correlated with the results obtained with macrophages *in vitro* whereas those observed for syn 14-1 did not. These results suggest that the interaction between syn 14-1 and macrophages would not be the only factor responsible for the up regulation of IL-6 as seen *in vivo*.

Taken into account that syn variants induced an altered profile of cytokines and that glycoprotein D (gD) has been pointed out as an inducer of TNF-α [7, 18] and IL-6 [19] during HSV-1 infection the sequence of this glycoprotein was analyzed. Results showed that both syn variants presented a point mutation in gD in comparison to parental virus. For syn14-1, the point mutation G889A induces a change D272N in the ectodomain of the mature form gD. In the case of syn17-2, the point mutation G1119T induces a change K348N in the cytoplasmic tail of the mature glycoprotein. Both mutations have not been previously reported and would not be present within the four functional regions of gD previously described [20].

As mentioned above, NK cells activity is also crucial for innate defenses. It has been demonstrated that NK cells are recruited to the airways early after HSV-1 infection restricting the early virus replication in the lung [21]. Moreover, Nandakumar *et al*., reported that NK cells activation by the virus contribute to the initial reduction in viral load enhancing the stimulatory ability of the dendritic cells by enabling effective antigen processing and presentation [22]. In order to study the proportion of NK cells and monocytes in lung tissue of mice infected with syn variants, eight-week-old BALB/c mice were infected intranasally with 10^6 PFU of HSV-1 F strain, syn14-1 or syn17-2 and lungs were dissected for flow cytometric analysis. The marker NK 1.1 was used to detect NK cells (APC Mouse Anti-Mouse NK-1.1, APC Mouse IgG2a κ, Isotype control); however, the antigen is also a marker for specialized population of T lymphocytes (NK-T cells); and CD11b was used to detect monocytes (FITC RAT Anti-Mouse CD11b, FITC Rat IgG2b, κ Isotype Control) (BD Pharmingen). As can be seen in Fig. 3 (white bars) no differences were observed in the percentage of monocytes present in lungs of mice infected with HSV-1 F and syn variants, either at 3 or 5 days p.i. The number of NK cells at 3 days p.i. was

similar for the three viruses (Fig. 3, grey bars), however, at 5 days p.i lower percentages of NK cells were observed for both HSV-1 F and syn17-2. Syn14-1 showed a significant increase in NK cells in comparison with mock infected mice (Fig. 3, grey bars). In order to address activation state of infiltrated cells, a combination of anti-NK 1.1 and anti-CD28 (PE Hamster Anti-mouse CD28, PE Hamster IgG2, λ1 Isotype Control, BD Pharmingen) antibodies were used to identify double positive cells as activated NK cells (NK+). As shown in Table 1, lungs of mice infected with HSV-1 F showed a similar percentage of NK+ cells at 3 and 5 days p.i. In contrast, more than 90% of NK were activated in lungs infected with syn14-1 both at 3 days p.i. and 5 days p.i. (near 65%). Finally, in lungs of mice infected with syn17-2 similar results to HSV-1 F were obtained at day 3 p.i. (71.9%), while at 5 days p.i. the percentage decreased. These results are in accordance with previous histopathological studies, in which infiltration of leukocytes was detected at day 5 p.i. in lungs of mice infected with syn14-1 [12]. On the contrary, for syn17-2 the lower levels of activated NK cells could be associated with the thickening of alveolar walls and the loss of morphology as previously reported [12]. Reading *et al.* demonstrated that cytotoxicity of lung NK cells is influenced by both NK number and their activation state [21]. Therefore, the severe effect observed in infections carried out with syn17-2 might be associated to the reduced levels of activated NK.

To summarize, the results presented in this paper suggest that syn variants have developed a strategy to delay the activation of macrophages and hence the release of proinflammatory cytokines. Thus, syn variants of HSV-1 could replicate and generate disease. In agreement with this hypothesis, the lower levels of mRNAs cytokines observed in *in vitro* experiments, would explain the early reduction of proinflamatory cytokines obtained for syn variants. Mogensen *et al.* reported that HSV-1 down regulates the production of several proinflammatory cytokines in a number of different cell types by mediating instability of proinflammatory cytokine mRNAs [4]. In this way, although alterations of gD in syn variants were detected in non-functional regions, it cannot be discarded that these modifications could be responsible, at least in part, for the altered proinflamatory cytokines pattern observed for syn variants. Nevertheless, it is important to note that the two variants proved to be avirulent when inoculated by intrvaginal route whereas both killed all

intranasally inoculated animals [12]. Therefore, the immune response at genital mucosa might be triggered in a different way comparing to the airway mucosa.

Clearence of HSV-1 infection requires a tightly coordinated interaction between innate and adaptive response. NK cells are the major cell type recruited to the airways early after HSV-1 infection [21], rapidly activated as demonstrated by the up-regulation of cytotoxic capacity and production of IFN-γ. Our results showed that although similar numbers of NK cells were detected at 3 days p.i. in lungs of mice inoculated with syn variants and parental virus, the level of activation was higher for NK cells isolated from animals infected with the syn variants. On the other hand, augmented levels of proinflamatory cytokines were also detected at 3 days p.i. for syn variants. It is important to consider that infectious virus could not be recorded from lungs of either HSV-1 or syn variants infected animals at this time. In this regard, it is tempting to speculate that NK cells are able to control viral replication but they are not required for an effective viral clearance. However, T cells in lungs play an important role in lowering viral loads. In fact, Adler *et al*. reported that mice deficient in NK and T cells were not able to survive after an intranasal infection, whereas mice only lacking T cells could survive [23]. In this line, the differential NK infiltration and/or activation observed for syn variants at 5 days p.i. would not be related to viral clearance but instead may be responsible for the injury observed in lungs of mice infected with the variants.

The ability of HSV to productively infect a wide range of hosts and cell types suggests that HSV has evolved to gain usage of alternative receptors and pathways to facilitate entry into multiple cell types [24]. Regardless of entry receptors or pathways utilized, HSV entry into host cell has common features among various routes of virus entry, including HSV fusion with the plasma membrane of the host cell. This indicates that HSV might recognize structural features of receptors that are conserved among various cell types. On this regard, it is tempting to speculate that the selective process exerted by carrageenans on HSV might generate viruses that retain their ability to infect a wide variety of cells and compensate a putative disadvantage at the stage of entrance with mutations at the level of TK and/or DNA pol genes [12, 25]. The panorama in vivo should contemplate also compensating mutations that would confer the virus the ability to evade the immune response.

In conclusion, our data provide evidence that the modulation of IL-6 and TNF-α seen *in vitro* as well as *in vivo* suggests that HSV-1 targets the proinflammatory host response as a mean of immune evasion. Results obtained in the present study are useful to understand the factors defining HSV-1 virulence in the respiratory tract of mice. Moreover, HSV-1 variants constitute a valuable tool to understand the immune systems and to investigate the contribution of specific components of mucosal immunity.

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References

[1] Finton KA, Strong RK. Structural insights into activation of antiviral NK cell responses. Immunol Rev. 2012; 250:239-257.

[2] Chew T, Taylor KE and Mossman KL. Innate and Adaptive Immune Responses to Herpes Simplex Virus. Viruses 2009; 1: 979-1002.

[3] Ellermann-Eriksen S. Macrophages and cytokines in the early defence against herpes simplex virus. Virol J. 2005 3; 2:59.

[4] Mogensen TH, Melchjorsen J, Malmgaard L, Casola A, Paludan SR. Suppression of proinflammatory cytokine expression by herpes simplex virus type 1. J Virol 2004;78:5883-5890.

[5] Lucey DR, Clerici M, Shearer GM. Type 1 and type 2 cytokine dysregulation in human infectious, neoplastic, and inflammatory diseases. Clin Microbiol Rev 1996;9:532-562.

[6] Romagnani S. The th1/th2 paradigm. Immunol Today 1997;18:263-266.

[7] Paludan SR, Mogensen SC. Virus-cell interactions regulating induction of tumor necrosis factor alpha production in macrophages infected with herpes simplex virus. J Virol 2001;75:10170-10178.

[8] Murphy EA, Davis JM, Brown AS, Carmichael MD, Ghaffar A, Mayer EP. Effect of il-6 deficiency on susceptibility to hsv-1 respiratory infection and intrinsic macrophage antiviral resistance. J Interferon Cytokine Res 2008;28:589-595.

[9] Opal SM, DePalo VA. Anti-inflammatory cytokines. Chest 2000;117:1162-1172.

[10] Stumpf TH, Case R, Shimeld C, Easty DL, Hill TJ. Primary herpes simplex virus type 1 infection of the eye triggers similar immune responses in the cornea and the skin of the eyelids. J Gen Virol 2002;83:1579-1590.

[11] Carlucci MJ, Scolaro LA, Damonte EB. Herpes simplex virus type 1 variants arising after selection with an antiviral carrageenan: Lack of correlation between drug susceptibility and syn phenotype. J Med Virol 2002;68:92-98.

[12] Mateu CG, Recalde MP, Artuso MC, Hermida G, Linero FN, Scolaro LA, Damonte EB, Pujol CA, Carlucci MJ. Emergence of herpes simplex virus-1 syncytial variants with altered virulence for mice after selection with a natural carrageenan. Sex Transm Dis 2011;38:555-561.

[13] Hull HF, Blumhagen JD, Benjamin D, Corey L. Herpes simplex viral pneumonitis in childhood. J Pediatr 1984;104:211-215.

[14] Smyth RL, Higenbottam TW, Scott JP, Wreghitt TG, Stewart S, Clelland CA, McGoldrick JP, Wallwork J. Herpes simplex virus infection in heart-lung transplant recipients. Transplantation 1990;49:735-739.

[15] Canalejo Castrillero E, Garcia Duran F, Cabello N, Garcia Martinez J. Herpes esophagitis in healthy adults and adolescents: Report of 3 cases and review of the literature. Medicine (Baltimore) 2010;89:204-210.

[16] Martinez E, de Diego A, Paradis A, Perpina M, Hernandez M. Herpes simplex pneumonia in a young immunocompetent man. Eur Respir J 1994;7:1185-1188.

[17] Glorieux S, Bachert C, Favoreel HW, Vandekerckhove AP, Steukers L, Rekecki A, Van den Broeck W, Goossens J, Croubels S, Clayton RF, Nauwynck HJ. Herpes simplex virus type 1 penetrates the basement membrane in human nasal respiratory mucosa. PLoS One 2011;6:e22160.

[18] Ankel H, Westra DF, Welling-Wester S, Lebon P. Induction of interferon-alpha by glycoprotein d of herpes simplex virus: A possible role of chemokine receptors. Virology 1998;251:317-326.

[19] Teng YC, Chen YT, Cheng YY, Hung SL. Requirements for the upregulation of interleukin-6 by herpes simplex virus-infected gingival fibroblasts. Viral Immunol 2005;18:170-178.

[20] Chiang HY, Cohen GH, Eisenberg RJ. Identification of functional regions of herpes simplex virus glycoprotein gd by using linker-insertion mutagenesis. J Virol 1994;68:2529-2543.

[21] Reading PC, Whitney PG, Barr DP, Smyth MJ, Brooks AG. Nk cells contribute to the early clearance of hsv-1 from the lung but cannot control replication in the central nervous system following intranasal infection. Eur J Immunol 2006;36:897-905.

[22] Nandakumar S, Woolard SN, Yuan D, Rouse BT, Kumaraguru U. Natural killer cells as novel helpers in anti-herpes simplex virus immune response. J Virol 2008;82:10820-10831.

[23] Adler H, Beland JL, Del-Pan NC, Kobzik L, Sobel RA, Rimm IJ. In the absence of t cells, natural killer cells protect from mortality due to HSV-1 encephalitis. J Neuroimmunol 1999;93:208-213.

[24] Karasneh GA, Shukla D. Herpes simplex virus infects most cell types in vitro: clues to its success. Virol J 2011; 8:481.

[25] Artuso C. Role of sulfated polysaccharides and herpes simples virus type 1 variants in viral pathogenicity. 2013. Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires. Doctoral Tesis.

Figure 1. Cytokines release and viral replication in mice intranasally infected. Ten (five for each time) female BALB/c mice were infected intranasally with $1x10^6$ UFP of HSV-1 F, syn14-1 and syn17-2. At 1 and 3 days p.i. three mice per group were sacrificed and BALs were performed for quantification of (a) TNF-α, (b) IL-6 and (c) IL-10 by ELISA. d) Viral titres were determined by plaque assay in homogenates of lungs from the other two mice of the group. * denotes a p-value < 0.05; ** denotes a p-value <0.05; # denotes a p-value <0.05.

Figure 2. Cytokine analysis in an *in vitro* model. RAW 264.7 murine macrophage cells were infected with HSV-1 F, syn14-1 and syn17-2 at a MOI of 10 PFU/cell. At different times p.i. supernatants and cell monolayers were harvested and IL-6 and TNF-α expression was evaluated by ELISA (a) and mRNAs synthesis of this cytokines was evaluated by RT-PCR assay (b). Band intensity was measured by using ImageJ program, and expressed as fold changing of the ratio between the respective cytokine and β-actin. The data shown are mean ± SD of two independent experiments. cc: cell control.

Figure 3. Cell influx in lungs of infected mice. Five Eight-week-old BALB/c mice per group were infected intranasally with $1x10^6$ UFP of HSV-1 F, syn14-1 and syn17-2. At 3 and 5 days p.i. lungs were harvested. (a) Cell influx was analyzed by flow cytometry and classified as monocytes ($CD11b^+$) and NK cells (NK 1.1^+). Histograms represent NK cells Isotype (grey), Mock (dashed line) and syn14-1 infection (solid line). No significant differences were detected with respect to control cells in the case of HSV-1 F and syn 17-2 histograms (data not shown) (b) Activated NK cells were classified as NK $1.1^+/CD28^+$. The data shown are mean ± SD of two independent experiments. * denotes a p-value < 0.05; ** denotes a p-value <0.05.

Table 1. Activated NK cells in lungs of infected mice. Five Eight-week-old BALB/c mice per group were infected intranasally with $1x10^6$ UFP of HSV-1 F, syn14-1 and syn17-2. At 3 and 5 days p.i. lungs were harvested. Activated NK cells were classified as NK $1.1^+/CD28^+$.

Glossary

Carrageenans: are a family of linear sulfated polysaccharides that are extracted from red seaweeds. They are widely used in the food industry, for their gelling, thickening and stabilizing properties.

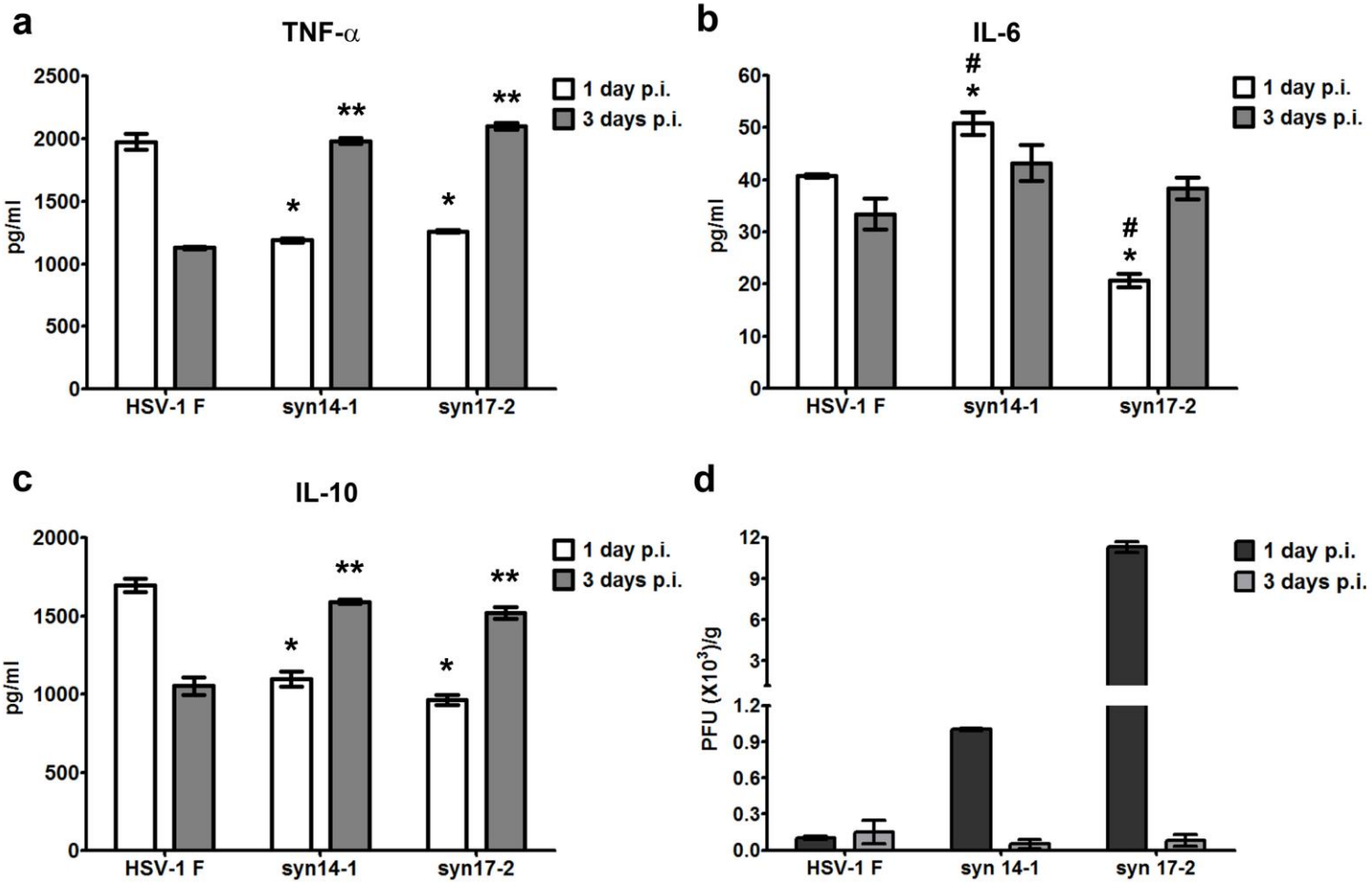
Selective pressure: It is a mutation-selective process used to obtain the variants. It consists of serial passages of virus *in vitro* in the presence of carrageenans.

Variants: mutants of virus obtained under selective pressure.

Cytokine: A small protein released by cells that has a specific effect on the interactions between cells, on communications between cells or on the behavior of cells. The cytokines includes the interleukins (as IL-6), lymphokines and cell signal molecules, such as tumor necrosis factor (TNF) and the interferons, which trigger inflammation and respond to infections. They are key molecules in modulating the immne response.

Syncytial: It is a multinucleate cell which can result from multiple cell fusions of uninucleated cells.

Virus	3 days p.i. (% cells ± SD)	5 days p.i. (% cells ± SD)
HSV-1 F	65.4 ± 1.9	70.6 ± 2.4
syn14-1	91.6 ± 3.6	95.0 ± 3.7
syn17-2	71.9 ± 2.1	43.9 ± 1.3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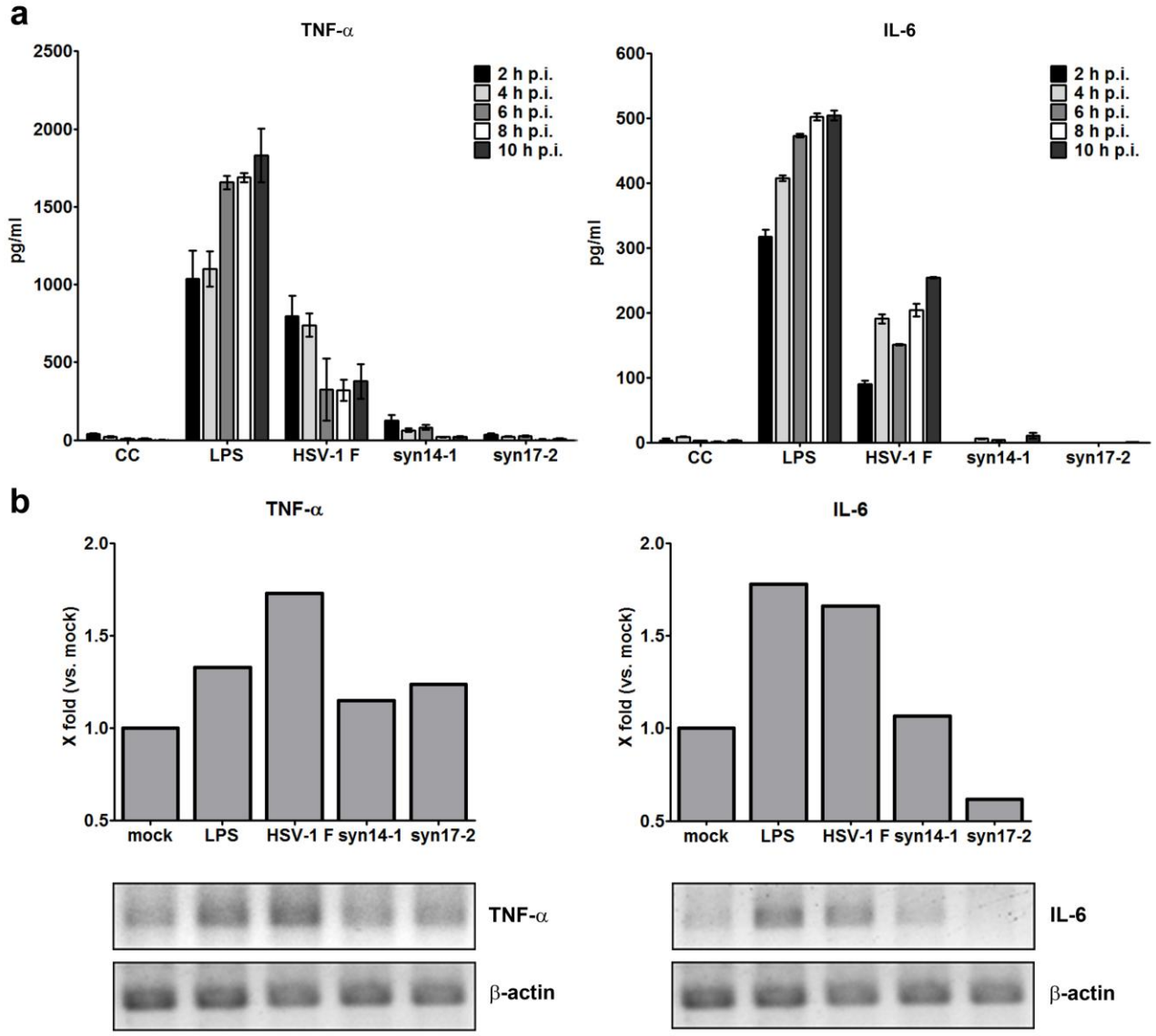
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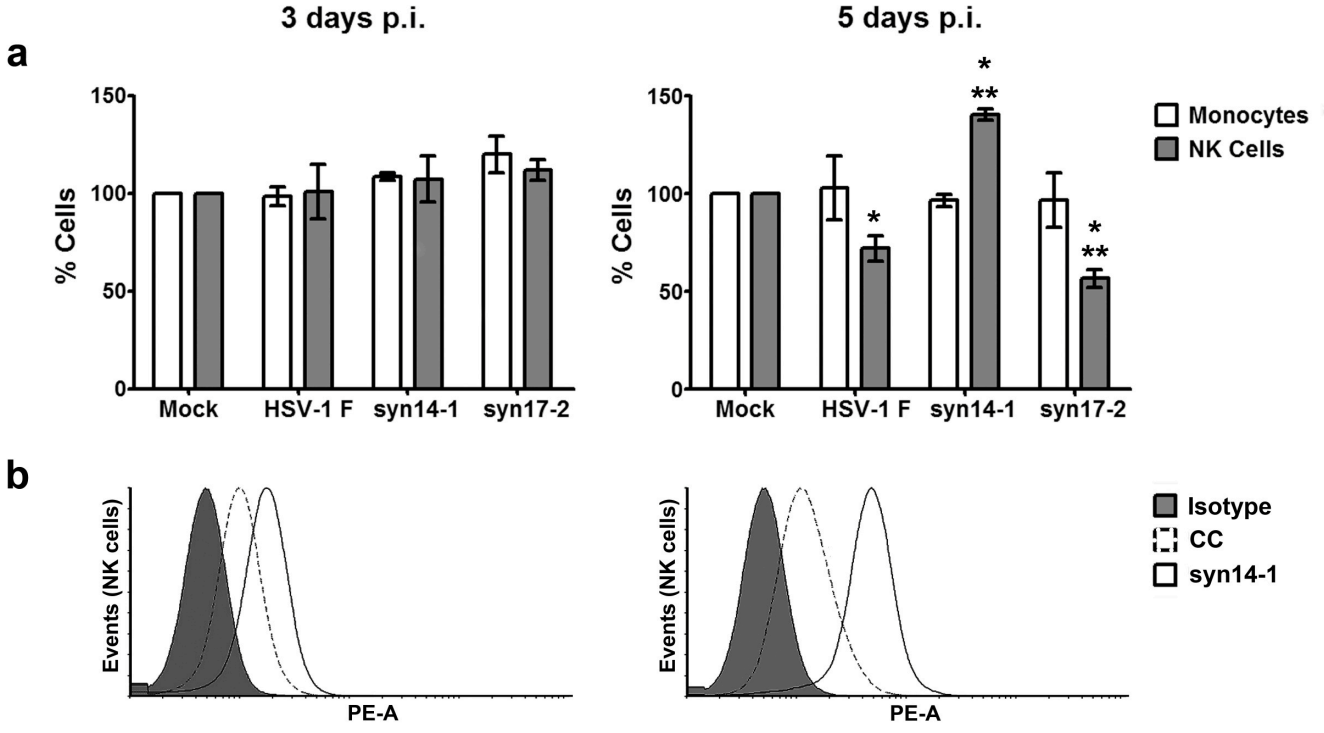
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Highlights

- HSV-1 syn variants arise during in vitro serial passages with carrageenans.
- The pathology of HSV-1 syn variants depends on modulation of the innate immune response activation.
- Low level of TNF-α is not due to an overproduction of antiinflammatory cytokine .
- The virulence of HSV-1 syn variants by intranasal route correlates with an enhanced replication.