JLB

Editorial

Editorial: **Macrophage heterogeneity and responses to influenza virus infection**

Suki M. Y. Lee,[*,†] Isabelle Dutry,*[†] and J. S. Malik Peiris*[*,†,1]

*Centre of Influenza Research and School of Public Health and †HKU-Pasteur Research Centre, The University of Hong Kong, Hong Kong, China

RECEIVED MARCH 12, 2012; REVISED APRIL 11, 2012; ACCEPTED APRIL 15, 2012. DOI: 10.1189/jlb.0312130

SEE CORRESPONDING ARTICLE ON PAGE 11

Macrophages play a central role in innate immune responses and are found in abundance in lungs of patients with fatal pneumonia caused by avian influenza H5N1. Respiratory epithelial cells, alveolar epithelial cells, and lung endothelial cells are targets for virus infection (reviewed in ref. [1]). It is therefore relevant to study influenza virus tropism, replication competence, and innate immune responses in physiologically relevant macrophages.

In this issue of *JLB*, Friesenhagen et al. [2] report a lack of productive virus replication (i.e., infectious virus released into cell culture supernatant) in macrophages infected in vitro with HPAI viruses H5N1 and H7N1 or seasonal influenza virus H1N1 (PR8). Furthermore, the HPAI H5N1 and H7N7 viruses elicited weaker innate immune responses compared with H1N1 (PR8). They suggest that the HPAI viruses evade innate immune responses, including type 1 IFN responses. These results differ in some respects with previous data.

Earlier studies comparing influenza A H1N1 and H5N1 virus infection in primary human MDMs showed that both viruses replicated productively in these cells and that HPAI H5N1 viruses induced a more potent proinflammatory response than seasonal H1N1 viruses [3, 4]. On the other hand, seasonal, pandemic 2009 and avian H5N1 influenza virus replication and proinflammatory cytokine responses were much poorer in AMs when compared with MDMs [5, 6]. AMs are resident in the lung and have a distinct phenotype induced by the lung microenvironment (interactions with epithelial cells, surfactant proteins, GM-CSF, etc.) [7].

What accounts for these differences? The conditions used to culture peripheral MDMs can polarize these cells into different phenotypes—the classically activated macrophages (M1) and the alternatively activated macrophages (M2). The concept of polarization and plasticity has been applied with more or less stringency to illustrate macrophage phenotypic variation in vitro and in vivo [7] and is still undergoing refinement. The classically activated macrophages are induced with GM-CSF and IFN-γ or LPS. They are proinflammatory and mediate a Th1 response. Alternatively activated macrophages mediate a Th2 response and are induced by differentiation in M-CSF in the presence of IL-4/IL-13 (M2a), immune complexes (M2b), or IL-10 (M2c) cells, each of them expressing different phenotypes in vitro. However, it is not clear if any of these macrophage subsets, differentiated from peripheral blood monocytes in vitro, accurately reflect the biological phenotype of AMs.

Studies on the interaction of influenza and macrophages in vitro [1–6] have used different culture conditions, which may shape their phenotype and polarization and thus, influence the ultimate outcome of the experiment (**Table 1**). Some of the factors that may affect the cell phenotype include the source and concentration of the serum used in the culture medium, the substrate used for culture (e.g., plastic, Teflon), and the addition of GM-CSF or IFN-γ to the culture medium. In some of these studies, macrophages were differentiated from peripheral blood-derived monocytes in culture medium with autologous human serum on a plastic substrate. Others have allowed the differentiation in the presence of GM-CSF. However, when such macrophages were compared directly with AMs derived from BAL, they differed in their permissiveness and response to influenza viruses [6].

Although HPAI H5N1 viruses in general appear to be more potent inducers of proinflammatory cytokines compared with seasonal influenza viruses in MDMs, there is variation between different H5N1 virus strains, and some H5N1 strains induce cytokines to a similar extent of seasonal influenza viruses [8, 10]. Furthermore, there is variation between donors in the intensity of the cytokine/chemokine response.

Some of these experimental factors may explain the differences in the re-

Abbreviations: AM=alveolar macrophage, HPAI=highly pathogenic avian influenza, MDM=monocyte-derived macrophage, PR8=A/Puerto Rico/8/34 virus

1. Correspondence: School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong Special Administrative Region, China. E-mail: malik@hku.hk

TABLE 1. Comparison of Relevant Studies Where Influenza Virus Infection of Macrophages Has Been Researched

Reference	Cheung et al . [3]	Yu et al. [5]	Perrone et al. [4]	van Riel et al. [6]
Source of cells	MDM from peripheral blood leukocytes	AM from human lung and MDM from peripheral blood leukocytes	MDM from peripheral blood leukocytes	AM from BAL and MDM from peripheral blood leukocytes
Culture conditions for MDM	Plastic dishes in RPMI medium with 5% autologous plasma differentiated for 14 days	Plastic dishes in RPMI medium with 5% autologous plasma differentiated for 14 days	Serum-free medium with 20% autologous plasma and GM-CSF differentiated for 7 days	Teflon flasks in RPMI with 5% human AB serum or 5% FCS with GM-CSF differentiated for 7 days
Virus strains used	H5N1: A/HK/483/97 A/HK/486/97 A/Vietnam/3212/04 H1N1: A/HK/54/98	H5N1: A/HK/483/97 H1N1: A/HK/54/98	H5N1: A/Thailand/16/04 A/Thailand/SP/83/04 H1N1: A/South Carolina/1/18 A/Texas/36/91	H5N1: A/Vietnam/1194/04 H3N2: A/NL/213/03 pH1N1: A/NL/602/09
Productive virus replication detected	Yes for all	AM: Yes for H5N1; marginal for H1N1 MDM: Yes for all	Yes for all	AM: No for all MDM: Yes for H3N2 and H5N1; no for pH1N1
Evidence for more potent cytokine/chemokine responses in H5N1 virus-infected cells	MDM: Yes	MDM: Yes AM: Equivocal Responses much less than seen in MDM	MDM (GM-CSF): Yes	MDM: Yes AM: No Responses much less than seen in MDM

pH1N1, Pandemic H1N1.

sults observed by Friesenhagen et al. [2] with others (Table 1). The strain of H5N1 virus A/Thailand/KAN-1/2004, which they use, has also been found by others to be a poor cytokine inducer [10]. The cytokine induction phenotype of HPAI H7N7 has not been investigated in detail by others, but our own unpublished data suggest that H7N7 viruses are not potent inducers of proinflammatory cytokines, which is in agreement with the findings of Friesenhagen and colleagues[2]. Their finding that H5N1, H7N7, and H1N1 fail to replicate productively in MDM differs from that of others and is more akin to what has been observed for some viruses in AMs. Differences in experimental strategy (e.g., virus inoculum not removed after infection of cells) may also contribute to the different conclusions by making it more difficult to detect low-level virus replication.

This review of influenza virus–macrophage interactions highlights the importance of defining the physiological state and relevance of the macrophages used for experimental study. It is clear that AMs differ dramatically from MDMs in their response to viral infection; they are generally less permissive to virus and release less innate immune mediators in response to infection. However, lungs of patients with severe influenza caused by viruses, such as H5N1, have massive infiltration with macrophage-like (CD68-positive) cells. Some of these cells are likely derived from infiltration of peripheral blood monocytes [7]. If so, are these newly infiltrated MDMs as docile as resident AMs, or do they retain the MDM phenotype? Which (if any) of the "alternatively activated" macrophages generated in vitro (see above) physiologically and phenotypically resemble AMs, lung interstitial macrophages, or newly infiltrating macrophages in the infected lung? The influenza-infected lung will have an altered cytokine and chemokine milieu, and what effect does this have on the resident AM and on newly recruited monocytes? It has been reported previously that macrophages may switch from one subset to another depending on their environment [7]. It has also been reported that cross-talk between innate immune mediators released from virus-infected macrophages can amplify and broaden cytokine responses in alveolar epithelial cells, thereby amplifying mediator cascades [1].

As Friesenhagen and colleagues [2] point out, alveolar epithelial cells and endothelial cells may be at least as, if not more pathophysiologically, important in this context. HPAI H5N1 viruses and seasonal viruses efficiently replicate

TABLE 1. (*continued*)

Sakabe et al. [8]	Monteerarat et al. [10]	Geiler et al. [9]	Friesenhagen et al. [2]
MDM from peripheral blood leukocytes	MDM from peripheral blood leukocytes	MDM from peripheral blood leukocytes	MDM from peripheral blood leukocytes
Medium with GM-CSF	RPMI with normal human serum and GM-CSF differentiated for 10 days	IMDM medium with 10% pooled human serum and GM-CSF differentiated for 14 days	Teflon bags in RPMI with 10% human AB serum differentiated for 7 days
H5N1: A/HK/483/97 A/VN/UT31203A/2007 A/VN/UT3028II/2003 A/IDN/UT3006/2005 A/Ck/Miyazaki/K11/2007 H1N1: A/Kawasaki/UTK-4/2009 H3N2: A/Kawasaki/UTK-20/2008 pH1N1: A/California/04/2009	H5N1: A range of clade 0, clade 1, clade 2.1, and clade 2.3.4 viruses used H1N1: Four viruses used H3N2: Three viruses used (see paper for details)	H5N1: A/Thailand/Kan-1/04 H1N1: A/New Caledonia/20/99 H3N2: A/California/7/04 pH1N1: A/HH/01/09	H5N1: A/Thailand/Kan-1/04 H1N1: PR8 H7N7: A/FPV/Bratislava/79
Yes for all; levels of replication differed among virus strains	Yes for all; levels of replication differed among virus strains	Yes for all	No for all
MDM (GM-CSF): Virus strain-dependent	MDM (GM-CSF): Virus strain-dependent	MDM (GM-CSF): Yes; H5N1 induced cytokines similar to H3N2 but much higher than H1N1 and pH1N1	MDM: No H1N1 induced cytokines much higher than H5N1 and H7N7

in type I and type II alveolar epithelial cells. In comparison with seasonal influenza viruses, HPAI H5N1 viruses are more potent inducers of IL-6, RANTES, MCP-1, and IFN-inducible protein 10 in these cells [1, 5]. HPAI H5N1, but not seasonal H1N1, productively replicated in polarized, differentiated lung microvascular endothelial cells [11, 12] and dysregulates innate immune responses [13]. There is a need for a systematic study of these complex interactions to understand their implications for disease pathogenesis.

ACKNOWLEDGMENTS

The authors' research has been supported by research grants from the University Grants Committee of the Hong Kong Special Administrative Region, China (Project No. AoE/M-12/06), and the U.S. National Institute of Allergy and Infectious Diseases (Contract HSN266200700005C).

REFERENCES

1. Peiris, J. S. M., Cheung, C. Y., Leung, C. Y., Nicholls, J.M., (2009) Innate immune responses to influenza A H5N1: friend or foe? *Trends Immunol.* **30,** 574–584.
2. Friesenhagen, J., Boergeling, Y., Hrincius, E., Ludwig, S., Roth, J., Viemann, D. (2012) Highly pathogenic avian influenza viruses inhibit effective immune responses of human blood derived macrophages. *J. Leukoc. Biol.* **92,** 11–20.
3. Cheung, C. Y., Poon, L. L., Lau, A. S., Luk, W., Lau, Y. L., Shortridge, K. F., Gordon, S., Guan, Y., Peiris, J. S. M. (2002) Induction of proinflammatory cytokines in human macrophages by influenza A (H5N1) viruses: a mechanism for the unusual severity of human disease? *Lancet* **360,** 1831–1837.
4. Perrone, L. A., Plowden, J. K., García-Sastre, A., Katz, J. M., Tumpey, T. M. (2008) H5N1 and 1918 pandemic influenza virus infection results in early and excessive infiltration of macrophages and neutrophils in the lungs of mice. *PLoS Pathog.* **4,** e1000115.
5. Yu, W. C., Chan, R. W., Wang, J., Travanty, E. A., Nicholls, J. M., Peiris, J. S. M., Mason, R. J., Chan, M. C. (2011) Viral replication and innate host responses in primary human alveolar epithelial cells and alveolar macrophages infected with influenza H5N1 and H1N1 viruses. *J. Virol.* **85,** 6844–6855.
6. Van Riel, D., Leijten, L. M., van der Eerden, M., Hoogsteden, H. C., Boven, L. A., Lambrecht, B. N., Osterhaus, A. D., Kuiken, T. (2011) Highly pathogenic avian influenza virus H5N1 infects alveolar macrophages without virus production or excessive TNF-α induction. *PLoS Pathog.* **7,** e1002099.
7. Gordon, S., Martinez, F. O. (2010) Alternative activation of macrophages: mechanism and functions. *Immunity* **32,** 593–604.
8. Sakabe, S., Iwatsuki-Horimoto, K., Takano, R., Nidom, C. A., Le, M. Q., Nagamura-Inoue, T., Horimoto, T., Yamashita, N., Kawaoka, Y. (2011) Cytokine production by primary human macrophages infected with highly pathogenic H5N1 or pandemic H1N1 2009 influenza viruses. *J. Gen. Virol.* **92,** 1428–1434.
9. Geiler, J., Michaelis, M., Sithisarn, P., Cinatl Jr., J. (2011) Comparison of pro-inflammatory cytokine expression and cellular signal transduction in human macrophages infected with different influenza A viruses. *Med. Microbiol. Immunol.* **200,** 53–60.
10. Monteerarat, Y., Sakabe, S., Ngamurulert, S., Srichatraphimuk, S., Jiamtom, W., Chaichuen, K., Thitithanyanont, A., Permpikul, P., Songserm, T., Puthavathana, P., Nidom, C. A., Mai le, Q., Iwatsuki-Horimoto, K., Kawaoka, Y., Auewarakul, P. (2010) Induction of TNF-α in human macrophages by avian and human influenza viruses. *Arch. Virol.* **155,** 1273–1279.
11. Chan, M. C., Chan, R. W., Yu, W. C., Ho, C. C., Chui, W. H., Lo, C. K., Yuen, K. M., Guan, Y., Nicholls, J. M., Peiris, J. S. M. (2009) Influenza H5N1 virus infection of polarized human alveolar epithelial cells and lung microvascular endothelial cells.

Respir. Res. **10,** 102. doi:10.1186/1465-9921-10-102.
12. Zeng, H., Pappas, C., Belser, J. A., Houser, K. V., Zhong, W., Wadford, D. A., Stevens, T., Balczon, R., Katz, J. M., Tumpey, T. M. (2012) Human pulmonary microvascular endothelial cells support productive replication of highly pathogenic avian influenza viruses: possible involvement in the pathogenesis of human H5N1 virus infection. *J. Virol.* **86,** 667–678.
13. Viemann, D., Schmolke, M., Lueken, A., Boergeling, Y., Friesenhagen, J., Wittkowski, H., Ludwig, S., Roth, J. (2011) H5N1 virus activates signaling pathways in human endothelial cells resulting in a specific imbalanced inflammatory response. *J. Immunol.* **186,** 164–173.

KEY WORDS:
alveolar macrophage · monocyte · lung · pathogenesis · H5N1 · cytokine

Editorial: **Is histamine the missing link in chronic inflammation?**

Ekaterini Tiligada[1]

Department of Pharmacology, Medical School, University of Athens, Athens, Greece

RECEIVED FEBRUARY 20, 2012; REVISED MARCH 24, 2012; ACCEPTED MARCH 25, 2012. DOI: 10.1189/jlb.0212093

SEE CORRESPONDING ARTICLE ON PAGE 21

Histamine elicits pleiotropic actions, largely through binding to four currently known GPCRs, designated as H_1R–H_4R, and it has been implicated in inflammation for over 80 years. H_1R and H_2R antagonists (antihistamines) attained blockbuster status for the treatment of allergy and gastrointestinal diseases, respectively, but they have proved to be significantly less effective or ineffective in chronic inflammation. The discovery of the H_3R and H_4R some years ago and their respective primary expression in the CNS and in hematopoietic cells revived the interest of the global scientific community and the pharmaceutical industry in histamine research and exposed attractive perspectives for the potential therapeutic exploitation of these new drug targets [1]. Importantly, a century after histamine was first linked to allergies, the identification of the H_4R at the turn of the millennium led to intense research over the last decade, which offered renewed hope that this is the missing link in tackling chronic inflammation [2] and even exposed additional roles for the "older" H_1Rs and H_2Rs. The consequences of the novel concept—that histamine exerts immunomodulatory actions in inflammation through H_4R signaling—and the potential exploitation of this activity for a range of the major, poorly treatable chronic inflammatory diseases are currently the subject of worldwide evaluation. Yet, our understanding of the functional mission of histamine in the multiple interconnected systems that constitute the immunological responses and inflammatory signals remains incomplete.

In this issue of the *Journal of Leukocyte Biology*, Gschwandtner et al. [3] explored the cross-talk of histamine, IL-27, and chemokine CXCL10 in an attempt to identify an essential regulatory pathway, which is critical for the pathogenesis of allergy and inflammatory skin diseases, such as chronic eczema and psoriasis. The authors showed that histamine selectively down-regulates the production of IL-27 in isolated human peripheral monocytes, whereas stimulation of skin keratinocytes with supernatants from these cell cultures down-regulates CXCL10 secretion (**Fig. 1**). In initial experiments, histamine reduced IL-27, but not IL-6, TNF-α, and IL-10 production, at mRNA and protein levels, at early rather than late timepoints, regardless of the TLR that drove monocyte activation. The functionality of the histamine-induced IL-27 down-regulation in monocytes was illustrated by the consequent decreased activation of keratinocytes. Although an underlying signaling mechanism was not revealed, the effects of histamine in reducing IL-27 production appeared not to rely on Stat1, Erk1/2, and NF-κB phosphorylation, despite the described regulation of these signaling molecules by histamine in other cell types [2]. Subsequent investigations focused on the identification of the type(s) of histamine receptors that mediated the response. With the use of selective, pharmacologically active agents, as well as bone marrow-derived DCs from BALB/c $H_4R^{-/-}$ mice, the authors document the orchestration of these concerted immunological responses by H_2R and H_4R.

The complexity of chronic inflammation-driven disorders is highlighted by the extensive literature on the interplay among the signals triggering inflammatory responses, the large repertoire of immune cell subsets and mediators shaping the phenotypic variations in inflamed tissues, and the downstream cascades underlying the initiation, propagation, and perpetuation of the response [4]. In particular, chronic inflammatory skin diseases are characterized by erythematous and pruritic skin lesions infiltrated by various cell types, including monocytes and T_H cells, eliciting the differentiation of specialized DC subsets through largely unexplored mechanisms [5]. The increased histamine levels in inflamed skin and the functional expression of histamine receptors on infiltrating immune cells, keratinocytes, and sensory neurons support the

Abbreviations: EU COST=European Union European Cooperation in Science and Technology, H_1R—H_4R=histamine H_1—H_4 receptor, $H_4R^{-/-}$=H_4R knockout mouse, IDEC=monocyte-derived inflammatory dendritic epidermal cell, Treg=regulatory T cell

1. Correspondence: Department of Pharmacology, Medical School, University of Athens, M. Asias 75, GR-11527, Athens, Greece. E-mail: aityliga@med.uoa.gr