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About the Cellular Tropism of the Gammaherpesvirus Bovine Herpesvirus Type 4

We read with interest the recent report by Egyed et al. (4) about the distribution in tissue, in the natural host, of bovine herpesvirus type 4 (BHV-4), a member of the gammaherpesvirus group. Using PCR, those authors were able to trace BHV-4 (Movar strain) in several tissues of experimentally infected cattle (mostly spleen, lung, trachea, and nasal epithelium tissues) at different times, ranging from 3 to 48 days postinfection. The authors also report consistent detection of BHV-4 DNA in what they consider to be a pure population of lymphocytes obtained from peripheral blood of the BHV-4-infected cattle, and then they describe how BHV-4 extensively replicated in these peripheral "lymphocytes" throughout the whole period of their study. According to Egyed et al. (4), their results would then prove that the target cell of this virus is the lymphocyte and not (or not only) macrophagic cells as we previously reported (8). Furthermore, Egyed et al. contend that their results on specific lymphocyte tropism of BHV-4 justify the classification of this bovine herpesvirus as a gammaherpesvirus closely related to Epstein-Barr virus (4). An analysis of the procedures used by these authors (4) and those referenced by them (1) lead us to conclude that, in spite of their plausible rationalization, their experimental results do not provide support for the notion that lymphocytes are the target cell for replication of BHV-4. The procedure used by these authors to isolate, starting from peripheral blood leukocytes, the cell fraction which they call lymphocytes consists of a classical protocol for mononuclear cell isolation by Ficoll-Hypaque centrifugation from human (1, 2) or cattle (3, 9) peripheral blood. Egyed et al. seem to overlook the fact that a mononuclear cell fraction obtained through Ficoll density gradient centrifugation of peripheral blood leukocytes of cattle and other mammals contains not only lymphocytes but also mononuclear phagocytic cells (monocytes) (2, 3, 9). Therefore, to state that those (wrongly assumed) "pure lymphocytes" are supporting BHV-4 replication only by the use of a highly sensitive technique such as PCR, without further providing additional proof of cell population purity by cell sorting or another cell analysis technique, is an oversight, if not a serious mistake. At the time we discovered the lymphoid association of BHV-4 during acute and persistent infection in cattle, we had already reported that the mononuclear cell fraction obtained by Ficoll-Hypaque centrifugation (density, 1.077 g/ml^{-}, precisely the same reagent used by Egyed et al.; Pharmacia, Upsala, Sweden) contained cells infected with BHV-4 during acute and persistent infection of cattle (6). Even when at that time we had some evidence for BHV-4 infectivity in adherent and nonadherent mononuclear cells, we could not precisely define the cell type(s) harboring BHV-4, due to the extreme paucity of BHV-4 in peripheral blood cells. Later on, using a laboratory model of splenic BHV-4 persistence, we unequivocally found that the only lymphoid cell associated with BHV-4 infectivity in the spleen was a non-T, non-B cell associated with macrophagic populations (8). Finally, and very importantly, a recent paper by us (5) confirmed the same findings with cattle, the natural host of BHV-4. Therefore, on the basis of results obtained with at least the most consistent site of BHV-4 lymphoid persistence (the spleen), we do not have evidence that lymphocytes are the target of BHV-4; instead, we have solid evidence that macrophagic cells are the site of BHV-4 persistence. The latter cells may be involved in the transportation of this virus in blood and lymph through migration inside mononuclear phagocytic cells (5–8). The fact that our findings support a rather unorthodox tropism for a gammaherpesvirus (i.e., BHV-4 using macrophages as a target cell rather than lymphocytes, a typical target for some gammaherpesviruses) should be viewed within the context of the evident divergence that exists between the currently favored classification of herpesviruses based on molecular structure and the actual biology of these viruses. The recent reclassification of Marek's disease virus, which biologically has always been a typical lymphotropic "gamma-type" herpesvirus, as an alphaherpesvirus (mainly on the basis of its genomic organization) is an excellent example of this apparent disagreement.

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Author's Reply

I thank Dr. Osorio and his colleagues for their reply to our paper on the distribution in tissue of BHV-4 in the natural host. Although our original goal in that work was not the study of the cell tropism of BHV-4 in the immune system, by PCR

analysis of blood leukocytes we nevertheless got involved with this problem.

Dr. Osorio is completely right that the supernatant on the Ficoll cushion consists of mononuclear cells (i.e., monocytes and lymphocytes) and that such a cell suspension cannot be considered a pure lymphocyte population, and we accept his opinion. That is why we should have used the word leukocytes instead of lymphocytes throughout the article.

Despite that, we do not feel that we made a serious mistake, for two reasons. First, the large majority of this cell suspension is surely lymphocytes. Supposing that only a small percentage of monocytes are BHV-4 infected and we diluted the sample 50 to 500 times, it is at least questionable where the positive PCR signal came from. During the last year, we concentrated our efforts on the cell tropism of BHV-4 within the immune system. We found clear evidence that BHV-4 replicates in B lymphocytes and has an inhibitory effect on some biological functions of these cells. On the basis of these results we used the word lymphocyte in the article, even if in that context it was not accurate. We intend to publish a manuscript about the cell tropism of BHV-4 in the immune system soon.

Dr. Osorio and coworkers found earlier that splenic macrophages and adherent and nonadherent blood leukocytes play a role in viral persistence (1, 2). In our opinion, our results do not contradict those of Dr. Osorio and colleagues. The game played between BHV-4 and the immune system is probably not as simple as researchers tend to believe.

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