Gag p27-Specific B- and T-Cell Responses in Simian Immunodeficiency Virus SIVagm-Infected African Green Monkeys

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Note

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Abstract

Nonpathogenic SIVagm infection of African green monkeys (AGMs) is characterized by the absence of a robust antibody response against Gag p27. To determine if this is accompanied by a selective loss of T cell responses to Gag p27, we studied $CD4^+$ and $CD8^+$ T cell responses against Gag p27 and other SIVagm antigens in the peripheral blood and lymph nodes of acutely- and chronically-infected AGMs. Our data show that AGMs can mount a T cell response against Gag p27, indicating that the absence of anti-p27 antibodies is not due to the absence of Gag p27-specific T cells.

Simian immunodeficiency virus (SIV) infection in African green monkeys (AGM) is nonpathogenic, even though it is characterized by plasma viral load (PVL) levels similar to those found during acute and chronic pathogenic infection of humans with HIV-1 and macaques with SIVmac (14). This feature is shared with other African non-human primates (NHP), such as sooty mangabeys and mandrills (19, 20). SIV-infected AGMs also display high viral loads in the gastrointestinal mucosa (11), a transient decline of circulating $CD4^+$ T cells during acute infection (13), and longer-lasting $CD4^+$ T cell depletion in the intestinal lamina propia (10). Concomitant with the peak viral load during acute infection, SIVagm-infected AGMs display transient increases of $CD4^+$ and $CD8^+$ T cells expressing activation and proliferation markers, such as MHC-II DR and Ki-67 (4, 13), and anti-SIVagm antibodies (Ab) are induced with kinetics similar to those found in SIVmac infection (5). Interestingly, however, the Ab response against Gag p27 is weak, if present at all (1, 2, 12, 15, 17, 18). This observation is surprising since, in the context of HIV-1 and SIVmac infections, Ab responses to Gag p27 are usually quite strong. Weak or low reactivity to Gag p27 has also been observed in some other natural SIV infections (7, 8, 20), but not in all (21). We wondered whether such a selective lack of Ab reactivity in the SIV-infected AGM might be related to a lack of Gag p27-specific T cells. With this hypothesis in mind, we first confirmed and extended the studies on humoral responses against Gag p27 by characterizing the antigen-specific IgG responses and mid-point titers against total SIVagm antigens (SIVagm virions) and recombinant Gag p27 (SIVagm) in naturally and experimentally SIVagm-infected AGMs. Second, we searched for the presence of Gag p27-specific T cell responses in SIVagm infection, by analyzing the $CD4^+$ and $CD8^+$ T cell responses specific for Gag p27 and other SIVagm proteins in blood and lymph nodes (LN) of acutely- and chronically-infected animals.

Humoral responses against SIV were analyzed in 50 wild-born AGMs (*Chlorocebus sabaeus*) and 17 Rhesus macaques (RM). The animals were housed at the Pasteur Institute in Dakar, Senegal, and the California National Primate Research Center, Davis, CA, respectively, according to institutional and national guidelines. RM were either non-infected (N=5) or intraveinously (I.V.) infected with SIVmac251 (N=12). AGMs were either non-infected (N=23), naturally infected (N=17) or I.V. infected with wild type SIVagm.sab92018 (N=10) (5, 9). IgG titers against SIVagm.Sab92018 virions or recombinant Gag p27 protein (rP27) were determined by ELISA using monkey anti-IgG as secondary antibodies (Fig. 1A and B). Virions had been purified by ultracentrifugation on a iodixanol cushion from cell-free supernatants of SIVagm.Sab92018-infected SupT1 cells. The His-tagged rP27 was constructed using DNA from gut cells of an SIVagm.sab92018-infected AGM (96011) (11). A Gag p27 PCR product was subcloned into pET-14b, and the recombinant protein was produced in BL21DE3pLysS *E. Coli* and purified on Ni-TA columns. SIV-infected macaques showed high IgG titers cross-reacting with both SIVagm virions (Fig. 1A and B, left) and rP27 (Fig. 1A and B, right). By contrast, only 2 out of 27 SIV-infected AGMs showed detectable IgG responses against rP27 (Fig. 1A and B, right), while 21 out of 27 displayed significant responses against SIVagm virions (Fig. 1A and B, left). Two AGMs out of 23 from the negative control group showed weak responses at the limit of detection against SIVagm and two against rP27, suggesting either natural response against SIVagm proteins, cross-reactivity with unknown pathogens, maternal antibodies or recent SIV-infection. Of note, the titers against whole SIV in the infected monkeys were higher in macaques than AGMs, may be due to lack of anti-p27 Ab in most AGMs.

The study of IgGs by Western blot using denatured SIVagm.sab92018 virions showed no or weak anti-Gag reponses in SIV-infected AGMs, yet the anti-Env responses were often strong (Fig. 1C). By contrast, SIV-infected macaques showed a dominant IgG cross-reactive response against the

SIVagm Gag p27 protein. Even if a response was detected more frequently than with the ELISA assays in AGMs, their magnitude was different and considerable weaker than in macaques.

To compare B and T cell responses over time, 5 STLV-seronegative AGMs were infected with SIVagm.sab92018 and the animals followed longitudinally during the acute and post-acute phase of infection until day 90 post-infection (p.i). Sequential blood samples were collected and biopsies of axillary and inguinal LNs were performed at day -5 and at three times p.i. (days 14, 43 and 62). PVL was measured by real time PCR (5). Since we searched for Gag p27-specific responses, we also quantified Gag p27 antigen in the plasma (SIV p27 antigen assay, Coulter, Miami, FL). Viral RNA and p27 antigenemia peaks were observed between days 7 and 14 p.i. (Fig. 2A and 2B, respectively). The Gag p27 levels were variable among the animals but in a range similar to those reported previously in AGMs and macaques (3, 5). As has also been observed in SIVmac infection (except for rapid progressors), plasma Gag p27 levels fell below the detection level in the post-acute phase (i.e., after day 28 p.i.) (Fig. 2B and *data not shown*). There were significant increases of circulating $CD8^+DR^+$ T cells at days 7 and 14 p.i., and of $CD8^+$Ki-67^+ T cells at days 14 and 28 p.i. (Fig. 2C and 2D, left panels). After day 28 p.i., the percentages were no more statistically different from baseline levels. In LN cells (LNCs), the percentage of $CD8^+$Ki-67^+ T cells rose from 3.1±1.1% before infection to 6.1±0.3% at day 62 p.i., but the difference was not statistically significant (Fig. 2D, right panel). The levels of blood $CD4^+DR^+$, $CD8^+DR^+$, and $CD8^+$Ki-67^+ T cells, and of LNC $CD8^+$ Ki-67^+ T cells, were positively correlated with viremia ($p=0.002$ for DR^+ cells and $p<0.02$ for Ki-67^+ cells). Altogether, these results confirm previous data showing early, transient T cell activation in the peripheral blood of SIVagm-infected AGMs (13).

We next looked for the presence of Ab responses against rP27 in these animals. No Ab were detected before infection. After infection, all five AGMs developed anti-SIVagm IgGs within 4 to

9 weeks p.-i., AGM2001 showing the fastest response (Fig.3A). While the humoral responses against whole virions were significant (Fig.3B, red line), the anti-rP27 responses were below the threshold for positivity (Fig.3B, green line), with the exception of one animal (AGM 02001). The anti-rp27 response in this animal was only transient since it was no more detectable at week 75 p.i., in contrast to the anti-SIV Ab that were sustained (Fig. 3B and *not shown*).

We next searched for T cell responses against Gag p27 as compared to other SIVagm antigens in these animals. Gag p27 epitopes were presented in two ways: in the context of rP27 and as synthetic peptides. The peptide pools (comprised of overlapping 15-mers) spanned the following SIVagm proteins: Gag p27, Gag without (w/o) p27, Env and Tat. The amino acid sequences of the Gag and Env peptides corresponded to the autologous wild type SIVagm.sab92018 sequence, and those of the Tat peptides to an SIVagm.sab consensus sequence. The latter was determined using Tat sequences of other SIVagm viruses from Senegal that are available in the databases (SIVagm.sab1c, SIVagm.sabD42 and SIVagm.sabD30). We measured T cell responses by investigating the antigen-induced proliferation. T cells from blood (PBMC) and LNs were analyzed. All assays were performed with fresh cells that were stimulated with 10 µg/ml of Gag rP27 and 5 µg/ml of peptides over a period of 4 days. Dead cells were gated out using 7-amino-actinomycin D (7-AAD) and dividing ($CFSE^{low}$) cells were analyzed after stimulation with media alone, SIV antigens or concanavalin A as a positive control. We detected significant Gag p27-specific proliferative responses for $CD8^+$ T cells in PBMC and for $CD4^+$ and $CD8^+$ T cells in LNCs (Fig. 3C). The animal with the detectable anti-p27 Ab (AGM 2001) did not show stronger p27-specific T cell responses than the other animals. Thus, all SIV-infected AGMs were able to mount a proliferative T cell response against p27, while anti-p27 IgG were lacking in four of

them. However, the SIVagm-specific T cell responses were detected at only a few time points post-infection.

We then analyzed the T cell responses in the chronic phase of naturally and SIVagm.sab92018-experimentally infected AGMs. PVL, peripheral blood cell counts ($CD4^+$ and $CD8^+$ T cells, $CD20^+$ B cells), and immune activation ($Ki\text{-}67^+CD8^+$ T cells) were similar in naturally-infected and in experimentally-infected AGMs (Fig. 4A). As expected, cell counts and immune activation levels were also not different from SIV negative AGMs (Fig. 4A). Again, we measured SIV-specific responses first by a proliferation assay (Fig. 4B). One out of five animals tested had a proliferative SIV-specific $CD4^+$ T cell response (against Gag w/o p27, Gag p27, Gag rP27, Env GP120 and Tat) and two had a $CD8^+$ T cell response (against Gag p27 in both and against Env GP120 and Tat in one). Two (one naturally-infected, one experimentally-infected with SIVagm.sab92018) did not show any detectable antigen-specific proliferative $CD4^+$ or $CD8^+$ T cell response.

These results were extended to an analysis of SIV-specific T cell cytokine responses, e.g., the production of IFN-γ and TNF-α in nine chronically-infected as compared to ten non-infected AGMs (Fig. 4C and D). Fresh cells were stimulated for eight hours with the antigens described above. SIV-specific cytokine responses were detected in $CD8^+$, but not in $CD4^+$ T cells. Seven animals out of nine showed a response against at least one antigen. The two animals showing no response were among the four naturally infected animals tested. We therefore cannot exclude that the absence of response in these two animals is due to the presence of highly divergent viruses. However, a precise epitope mapping in SIVagm sequences would be necessary to confirm this. In those animals showing a SIVagm-specific cytokine T cell response, the latter were directed against Gag p27 (4 out of 9 animals), other Gag proteins than p27 (2 out of 9 animals), and Env GP120 (4 out of 9 animals). In the experimentally infected animals, we might have

underestimated the responses against Tat as compared to Gag and Env antigens, since the Tat peptides corresponded to an SIVagm.sab consensus sequence and not to the autologous virus (SIVagm.Sab92018). There was no correlation between the magnitude or breath of SIV-specific T cell responses and immune activation or VL.

Altogether, our study demonstrates that AGMs can mount T cell proliferative and cytokine responses against Gag p27. The T cell response was variable among the animals. In general, it appeared moderate, comparable to chronically SIV-infected RM (9). Of note, T cell responses were not consistently detected at all time points and not in all animals. We cannot exclude the possibility that we underestimated the magnitude of the cytokine responses. For instance, we did not co-stimulate the cells during the assays. However, cytokine responses were also variable in vervet AGMs with a trend for lower levels as compared to RMs even by using more sensitive assays (23). In sooty mangabeys, the responses were also reported to be not stronger than in RM. This is in line with the lack of efficient control of viral replication in natural hosts (6, 22).

In our study we show that IgG responses against Gag p27 are either lacking, weak or transient, while Ab against other SIVagm proteins are present. The mechanisms underlying this selective lack of Gag p27 Ab responses are unclear. It could be related to a moderate and/or dysfunctional $CD4^+$ T cell responses and/or due to an unknown suppressive regulatory mechanism. SIV-specific T cell cytokine responses were indeed principally found at the $CD8^+$ T cell level. This was also reported in SIVsm-infected sooty mangabeys (SM) (6, 22). Here, we also searched for SIVagm Gag p27-specific proliferative responses. Interestingly, they were detected for $CD4^+$ T cells indicating the presence of p27-specific $CD4^+$ memory cells in AGMs. Moreover, AGMs can potentially mount a strong and sustained anti-Gag p27 humoral response, when appropriately immunized (*D. Favre et al., manuscript in preparation*). This suggests that there is no central B cell tolerance against p27 Gag protein in AGMs, nor an inherent inability for $CD4^+$ T cells to

provide helper B cell functions. The transient nature of anti-p27 Ab in one animal would be in favor of regulatory mechanisms, but that needs to be confirmed. Another explanation could be that AGMs are able to mount Ab responses against some p27 epitopes but not to those exposed by the native protein, which would explain why we and others detect more frequently humoral responses in WB than in Elisa assays (16).

In conclusion, we characterized the IgG responses against SIVagm and confirmed a lower humoral response against p27 than in RM. Moreover, our study reveals that cytokine and proliferative T cell responses against SIVagm Gag p27 are detectable in AGMs. Thus, the reduced ability of the AGM to produce antibodies against Gag p27 post-infection is not related to a lack of Gag p27-specific T cells.

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REFERENCES

1. **Allan, J. S., P. Kanda, R. C. Kennedy, E. K. Cobb, M. Anthony, and J. W. Eichberg.** 1990. Isolation and characterization of simian immunodeficiency viruses from two subspecies of African Green Monkeys. AIDS Res. Hum. Retroviruses **6:**275-285.
2. **Allan, J. S., M. Short, M. E. Taylor, S. Su, V. M. Hirsch, P. R. Johnson, G. M. Shaw, and B. H. Hahn.** 1991. Species-specific diversity among simian immunodeficiency viruses from African green monkeys. J. Virol. **65:**2816-2828.
3. **Chakrabarti, L., M.-C. Cumont, L. Montagnier, and B. Hurtrel.** 1994. Variable course of primary simian immunodeficiency virus infection in lymph nodes: relation to disease progression. J. Virol. **68:**6634-6643.
4. **Cumont, M. C., O. Diop, B. Vaslin, C. Elbim, L. Viollet, V. Monceaux, S. Lay, G. Silvestri, R. Le Grand, M. Muller-Trutwin, B. Hurtrel, and J. Estaquier.** 2008. Early divergence in lymphoid tissue apoptosis between pathogenic and nonpathogenic simian immunodeficiency virus infections of nonhuman primates. J Virol **82:**1175-84.
5. **Diop, O. M., A. Gueye, M. Dias-Tavares, C. Kornfeld, A. Faye, P. Ave, M. Huerre, S. Corbet, F. Barre-Sinoussi, and M. C. Muller-Trutwin.** 2000. High levels of viral replication during primary simian immunodeficiency virus SIVagm infection are rapidly and strongly controlled in African green monkeys. J Virol **74:**7538-47.
6. **Dunham, R., P. Pagliardini, S. Gordon, B. Sumpter, J. Engram, A. Moanna, M. Paiardini, J. N. Mandl, B. Lawson, S. Garg, H. M. McClure, Y. X. Xu, C. Ibegbu, K. Easley, N. Katz, I. Pandrea, C. Apetrei, D. L. Sodora, S. I. Staprans, M. B. Feinberg, and G. Silvestri.** 2006. The AIDS resistance of naturally SIV-infected sooty mangabeys is independent of cellular immunity to the virus. Blood **108:**209-17.
7. **Emau, P., H. M. McClure, M. Isahakia, J. G. Else, and P. N. Fultz.** 1991. Isolation from African Sykes Monkeys (Cercopithecus mitis) of a lentivirus related to human and simian immunodeficiency viruses. J. Virol. **65:**2135-2140.
8. **Fultz, P. N., R. B. Stricker, H. M. McClure, D. C. Anderson, W. M. Switzer, and C. Horaist.** 1990. Humoral response to SIV/SMM infection in macaque and mangabey monkeys. J Acquir Immune Defic Syndr **3:**319-29.
9. **Genesca, M., P. J. Skinner, J. J. Hong, J. Li, D. Lu, M. B. McChesney, and C. J. Miller.** 2008. With minimal systemic T-cell expansion, CD8+ T Cells mediate protection of rhesus macaques immunized with attenuated simian-human immunodeficiency virus SHIV89.6 from vaginal challenge with simian immunodeficiency virus. J Virol **82:**11181-96.
10. **Gordon, S. N., N. R. Klatt, S. E. Bosinger, J. M. Brenchley, J. M. Milush, J. C. Engram, R. M. Dunham, M. Paiardini, S. Klucking, A. Danesh, E. A. Strobert, C. Apetrei, I. V. Pandrea, D. Kelvin, D. C. Douek, S. I. Staprans, D. L. Sodora, and G. Silvestri.** 2007. Severe depletion of mucosal CD4+ T cells in AIDS-free simian immunodeficiency virus-infected sooty mangabeys. J Immunol **179:**3026-34.

11. **Gueye, A., O. M. Diop, M. J. Ploquin, C. Kornfeld, A. Faye, M. C. Cumont, B. Hurtrel, F. Barre-Sinoussi, and M. C. Muller-Trutwin.** 2004. Viral load in tissues during the early and chronic phase of non-pathogenic SIVagm infection. J Med Primatol **33:**83-97.
12. **Kanki, P. J., J. Alroy, and M. Essex.** 1985. Isolation of T-lymphotropic retrovirus related to HTLV-III/LAV from wild-caught African green monkeys. Science **230:**951-4.
13. **Kornfeld, C., M. J. Ploquin, I. Pandrea, A. Faye, R. Onanga, C. Apetrei, V. Poaty-Mavoungou, P. Rouquet, J. Estaquier, L. Mortara, J. F. Desoutter, C. Butor, R. Le Grand, P. Roques, F. Simon, F. Barre-Sinoussi, O. M. Diop, and M. C. Muller-Trutwin.** 2005. Antiinflammatory profiles during primary SIV infection in African green monkeys are associated with protection against AIDS. J Clin Invest **115:**1082-91.
14. **Muller, M. C., and F. Barre-Sinoussi.** 2003. SIVagm: genetic and biological features associated with replication. Front Biosci **8:**d1170-85.
15. **Müller, M. C., N. K. Saksena, E. Nerrienet, C. Chappey, V. M. A. Hervé, J.-P. Durand, P. Legal-Campodonico, M.-C. Lang, J.-P. Digoutte, A. J. Georges, M.-C. Georges-Courbot, P. Sonigo, and F. Barré-Sinoussi.** 1993. Simian immunodeficiency viruses from Central and Western Africa: evidence for a new species-specific lentivirus in tantalus monkeys. J. Virol. **67:**1227-1235.
16. **Norley, S., and R. Kurth.** 2004. The role of the immune response during SIVagm infection of the African green monkey natural host. Front Biosci **9:**550-64.
17. **Norley, S. G., G. Kraus, J. Ennen, J. Bonilla, H. König, and R. Kurth.** 1990. Immunological studies of the basis for the apathogenicity of simian immunodeficiency virus from African green monkeys. Proc. Natl. Acad. Sci. USA **87:**9067-9071.
18. **Ohta, Y., T. Masuda, H. Tsujimoto, K. Ishikawa, T. Kodama, S. Morikawa, M. Nakai, S. Honjo, and M. Hayami.** 1988. Isolation of simian immunodeficiency virus from African green monkeys and seroepidemiological survey of the virus in various non-human primates. Int. J. Cancer **41:**115-122.
19. **Onanga, R., C. Kornfeld, I. Pandrea, J. Estaquier, S. Souquiere, P. Rouquet, V. P. Mavoungou, O. Bourry, S. M'Boup, F. Barre-Sinoussi, F. Simon, C. Apetrei, P. Roques, and M. C. Muller-Trutwin.** 2002. High levels of viral replication contrast with only transient changes in CD4+ and CD8+ cell numbers during the early phase of experimental infection with SIVmnd-1 in Mandrillus sphinx. J Virol **76:**10256-63.
20. **Rey-Cuille, M. A., J. L. Berthier, M. C. Bomsel-Demontoy, Y. Chaduc, L. Montagnier, A. G. Hovanessian, and L. A. Chakrabarti.** 1998. Simian immunodeficiency virus replicates to high levels in sooty mangabeys without inducing disease. J Virol **72:**3872-86.
21. **Santiago, M. L., C. M. Rodenburg, S. Kamenya, F. Bibollet-Ruche, F. Gao, E. Bailes, S. Meleth, S. J. Soong, J. M. Kilby, Z. Moldoveanu, B. Fahey, M. N. Muller, A. Ayouba, E. Nerrienet, H. M. McClure, J. L. Heeney, A. E. Pusey, D. A. Collins, C. Boesch, R. W. Wrangham, J. Goodall, P. M. Sharp, G. M. Shaw, and B. H. Hahn.** 2002. SIVcpz in wild chimpanzees. Science **295:**465.
22. **Wang, Z., B. Metcalf, R. M. Ribeiro, H. McClure, and A. Kaur.** 2006. Th-1-type cytotoxic CD8+ T-lymphocyte responses to simian immunodeficiency virus (SIV) are a consistent feature of natural SIV infection in sooty mangabeys. J Virol **80:**2771-83.
23. **Zahn, R. C., M. D. Rett, B. Korioth-Schmitz, Y. Sun, A. P. Buzby, S. Goldstein, C. R. Brown, R. A. Byrum, G. J. Freeman, N. L. Letvin, V. M. Hirsch, and J. E. Schmitz.**

2008. Simian Immunodeficiency Virus (SIV)-Specific CD8+ T-Cell Responses in Vervet African Green Monkeys Chronically Infected with SIVagm. J Virol **82:**11577-88.

FIGURE LEGENDS

Figure 1: Cross-sectional analysis of IgG antibody responses against SIVagm or Gag p27 in SIV-infected AGMs and RMs.

(**A and B**) Cross-sectional analysis by ELISA. IgG antibodies against SIVagm.sab$_{92018}$ virions or recombinant p27-Gag antigens were determined in SIV negative and chronically SIVmac$_{251}$-infected RMs and in SIV negative and chronically SIVagm-infected AGMs, that were either naturally or experimentally-infected with SIVagm.sab$_{92018}$. Antibody titers were calculated for each animal by limited dilution of plasma on coated ELISA plates with 5µg/ml of (p27 equivalent) virions (left) or 1µg/ml of the monomeric recombinant protein (rP27) (right). IgG detection by ELISA displayed high background for rP27, especially at the highest plasma concentration (e.g. 1/100 and 1/400 plasma dilution) in SIV-negative RM and AGMs. To discriminate between positive responses and background, calculated dose-response curves were compared to theoretical sigmoid-dose response curves corresponding to the 95% confidence interval of SIV-negative animals. By convention, responses were considered as background when sigmoid dose-response curves were graphically within the 95% confidence interval of SIV negative animals, and when the calculated -LogEC50 was lower than the upper theoretical sigmoid dose-response curve from SIV negative animals (corresponding to a threshold of –logEC50 = 2.8). **(A)** Results (O.D. 450) are represented for both virions (left) and rP27 (right) over plasma dilution (log10) on a per animal basis (data points) and for each group (lines). Lines represent the sigmoid dose-response curves for each group (Prism 4, Graphpad) (**B**) Mid-point IgG titers were determined for each animal from individual sigmoid dose-response curves, and presented as the log10 value from the reciprocal of the effective concentration that corresponds to 50% response between minimum and maximum O.D. 450 (-log EC50). Horizontal bars represent

the median mid-point titer per each group. Man-Whitney non-parametric tests were applied for statistical analysis (n.s., non significant with p values > 0.1) (**C**) Cross-sectional analysis of Ab against SIVagm proteins by Western blot using denatured SIVagm.sab$_{92018}$. For the positive controls on the left, we used sera from an SIVmac$_{251}$-infected macaque and a SIVagm.sab$_{92018}$ infected AGM. Development times and reagents were identical for all western blots. Mo: months of infection, y= years of infection, C-: negative control, and C+ : positive control.

Figure 2. Plasma viremia and T cell activation in blood and lymph nodes of five longitudinally-followed SIVagm.sab$_{92018}$-infected African green monkeys. (A) SIVagm.sab RNA copy numbers in plasma; **(B)** Plasma Gag p27 concentrations; **(C)** Percentages of MHC-II DR positive CD4$^+$ (●) and CD8$^+$ (○) T cells within, respectively, total CD4$^+$ and CD8$^+$ T cells from PBMC and LNCs; **(D)** Percentages of Ki-67 positive CD4$^+$ (●) and CD8$^+$ (○) T cells within, respectively, total CD4$^+$ and CD8$^+$ T cells from PBMC and LNCs. Results are shown as the mean ± SEM. Asterisks indicate statistically significant differences as compared to levels before infection ($p < 0.05$).

Figure 3. Longitudinal analysis of IgG titers and T cell proliferative responses against SIVagm and Gag p27 in five SIVagm.sab$_{92018}$-experimentally infected AGMs. (A+B) Ab responses were analyzed by ELISA. (A) IgG dose-response curves against SIVagm (upper) and rP27 (lower) are shown over time (week -1 to week +24 p.i.). (B) Mid-point titers were calculated as described in Figure 1A. Continuous lines correspond to median titers from all 5 animals (red, anti-SIVagm IgGs; green, anti-p27 IgGs). (C) Proliferative responses of CD4$^+$ and CD8$^+$ T cells were assessed by flow cytometry, using CFSE staining. CD4$^+$ and CD8$^+$ T cell responses in PBMCs (left) and LNCs (right) after stimulation with peptide pools (Gag w/o p27, P27, and Tat)

and Gag rP27 are shown for each animal. All data are reported after background subtraction. Results are presented in columns as mean ± SEM. Asterisks indicate statistically significant differences compared to individual values before infection ($p<0.05$).

Figure 4. Immune parameters and SIVagm-specific proliferative and cytokine T cell responses in chronically-infected AGMs. (A) Cell counts ($CD4^+$ and $CD8^+$ T cells, B cells) , and immune activation levels (% of Ki-67^+ in $CD8^+$ T cells) in SIVagm-naturally infected AGMs (n=4) and SIVagm.sab_{92018}-experimentally infected AGMs (n=6) compared to uninfected AGMs (n=10). PVL if known is indicated. Green, blue and orange symbols correspond, respectively, to non-infected, naturally infected and experimentally infected AGMs. (**B**) Proliferative response to SIVagm antigens in chronically-infected AGMs (n=5) compared to uninfected AGMs (n=3). PBMCs were stimulated with the same antigens as described in Fig. 3. (**C**) Analysis of cytokine responses (IFN-γ and TNF-α) by SIVagm-specific T cells. ConA was used as a positive control. Representative results from a single animal are shown here. **(D)** Cumulative values of SIVagm-specific TNF-α and IFN-γ responses in chronically-infected animals. The responses to SIVagm antigens were analyzed in peripheral blood specimens of 4 naturally- and 5 experimentally-infected AGMs, as well as 10 uninfected AGMs. The data are reported after background subtraction corresponding to the subtraction of the frequency of positive events from the unstimulated samples to the frequency of positive events from the antigen-specific stimulation. Proliferative T cell responses and cytokine T cell responses in SIV-infected AGMs were defined as positive when higher than the mean + 3St.Dev of the responses from uninfected animals.

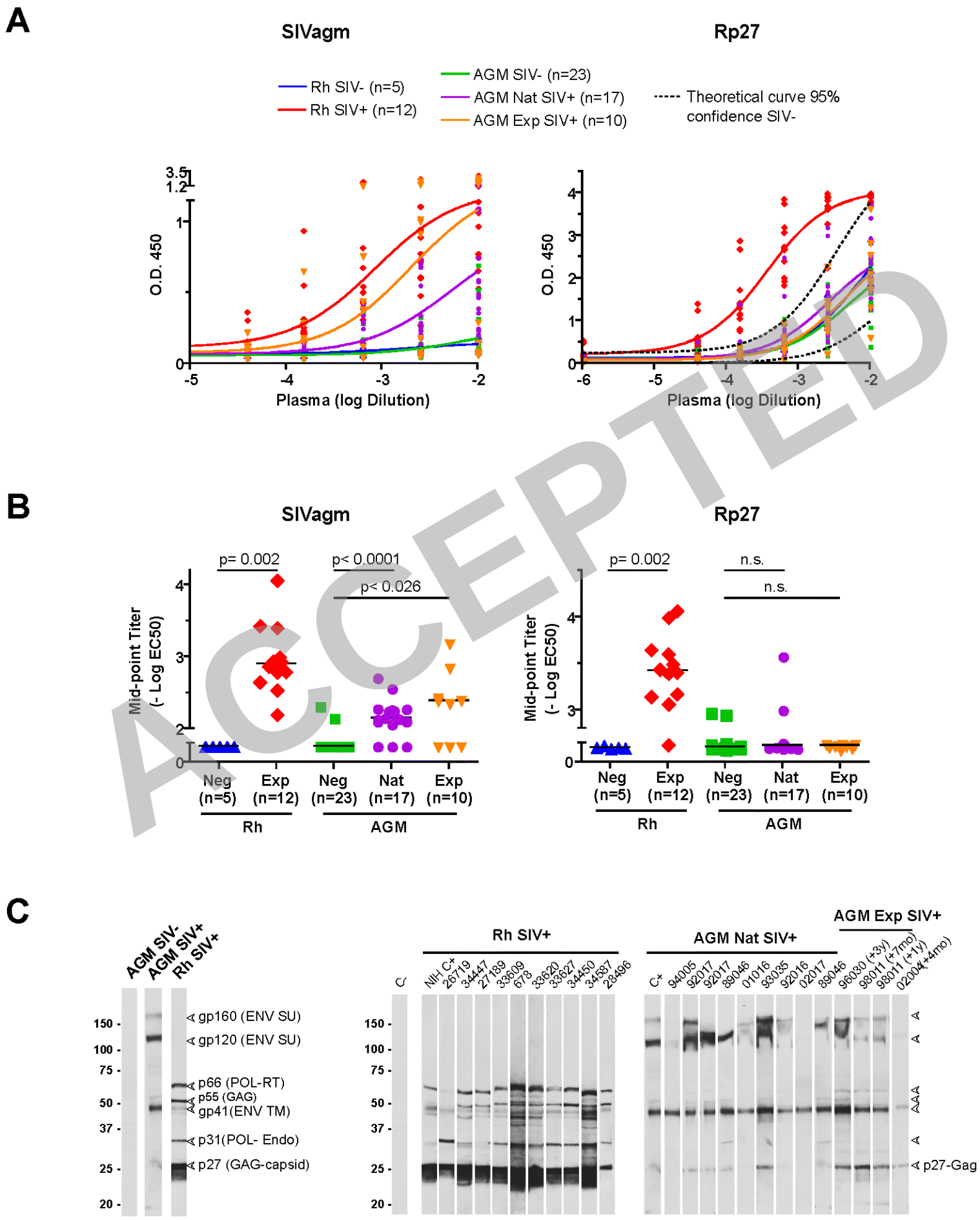


Figure 1

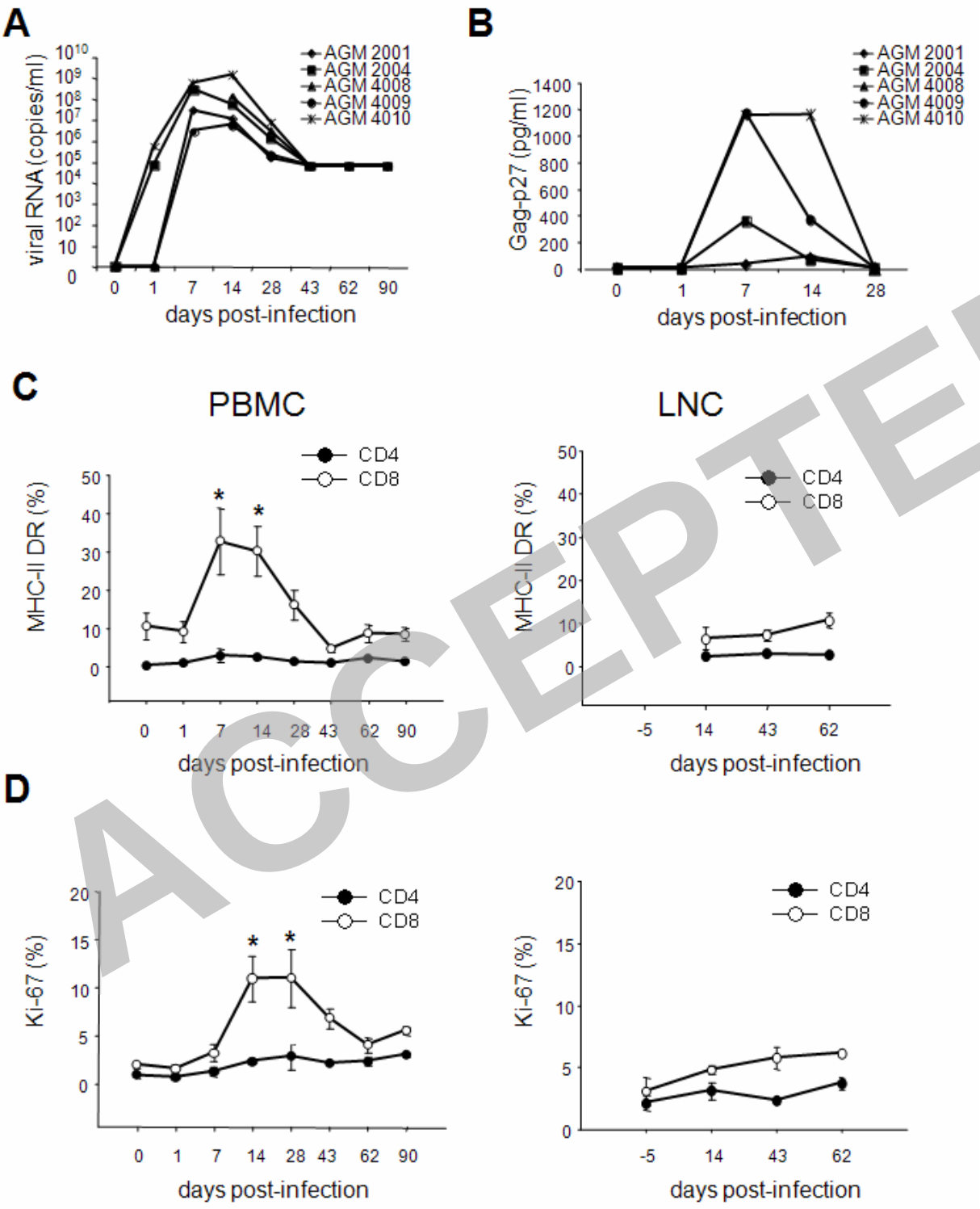
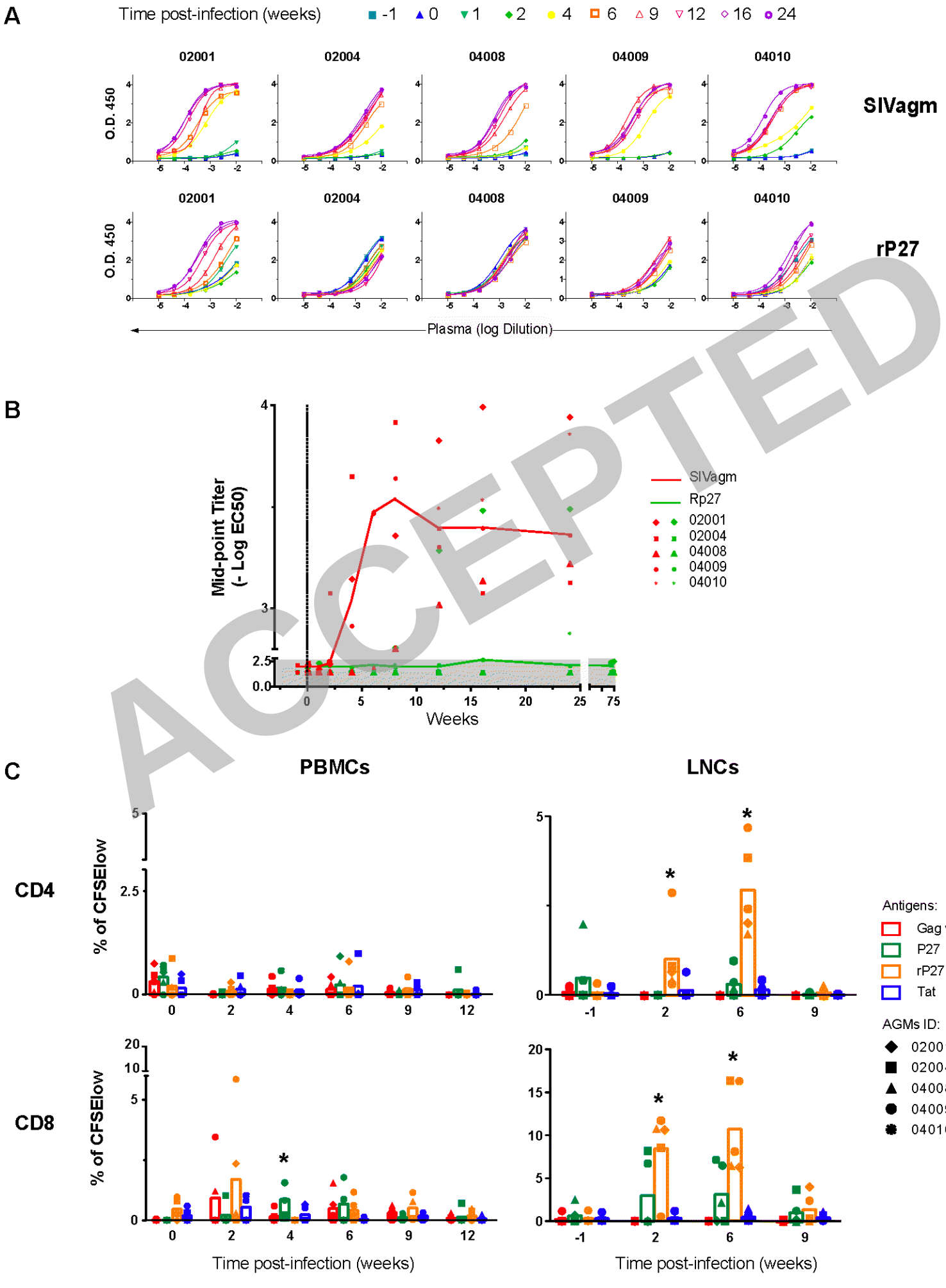


Figure 2



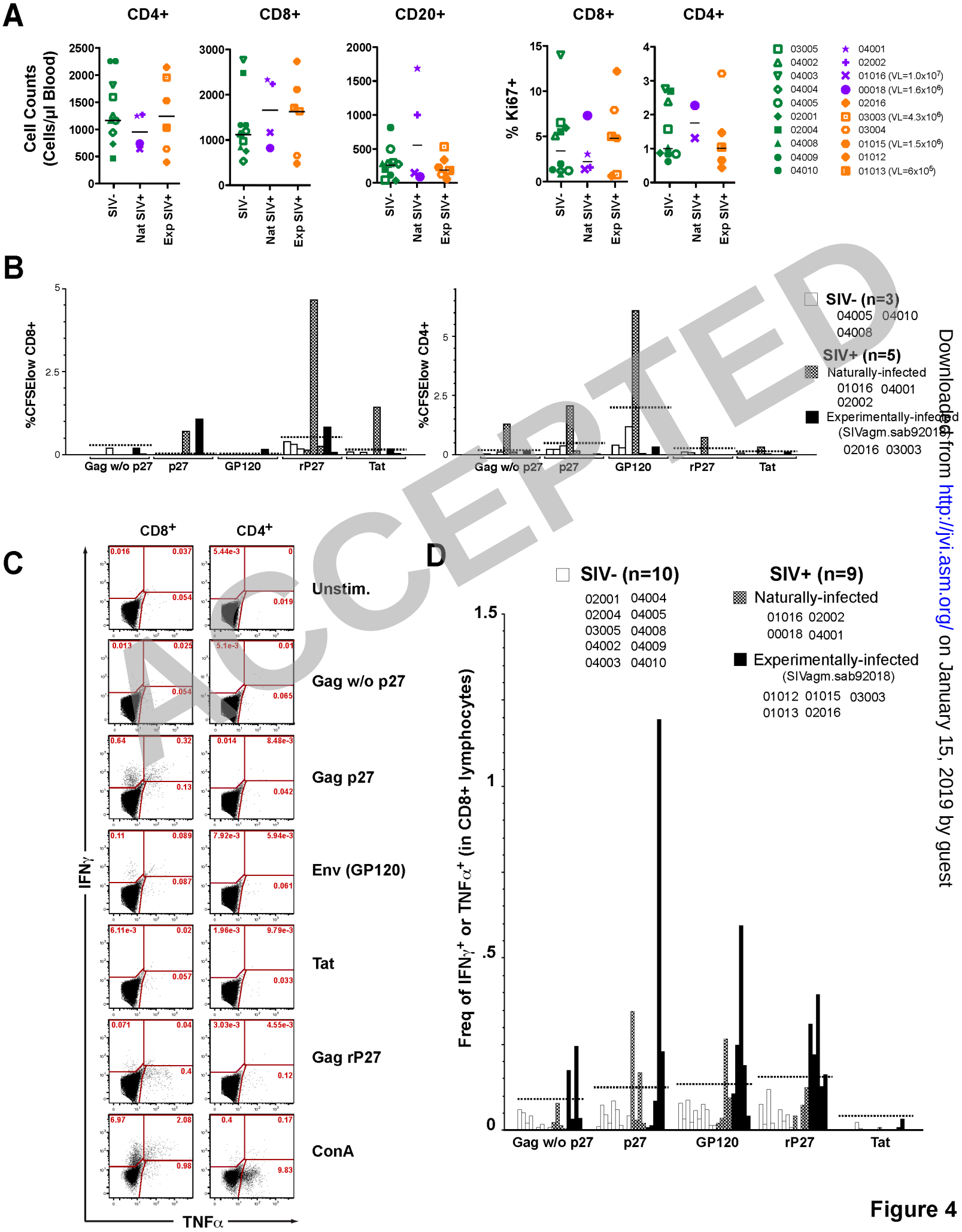


Figure 4