SUPPORTING INFORMATION FOR:

Treatment with IL-7 Prevents the Decline of Circulating CD4\(^+\) T Cells during the Acute Phase of SIV Infection in Rhesus Macaques

Lia Vassena,\(^1,2\) Huiyi Miao,\(^1\) Raffaello Cimbro,\(^1\) Mauro S. Malnati,\(^2\) Giulia Cassina,\(^2\) Michael A. Proschan,\(^3\) Vanessa M. Hirsch,\(^4\) Bernard A. Lafont,\(^5\) Michel Morre,\(^6\) Anthony S. Fauci,\(^1\) and Paolo Lusso\(^1\)

\(^1\)Laboratory of Immunoregulation, \(^3\)Biostatistics Research Branch and \(^4\)Laboratory of Molecular Medicine, \(^5\)Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892; \(^2\)Human Virology Unit, DIBIT-HSR, 20132 Milano, Italy; and \(^6\)Cytheris, Issy-les Moulineaux 92370, France

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SUPPLEMENTARY METHODS

MHC screening and study groups
A critical issue that we considered in the study design was the assignment of the 12 animals to the two study groups (i.e., IL-7 treatment and control) in order to avoid potential biases in SIV-disease susceptibility. Since previous work identified MHC class-I alleles with disease/viral load-protective (Mamu-A01, Mamu-A08, Mamu-B08) or -enhancing (Mamu-B01) properties [1-4], we pre-screened the animals for defined Mamu haplotypes. A positive score (+1) was attributed to the presence of Mamu-A01, A08 or B08; a negative score (-1) to the presence of Mamu-B01 (Table S2). The final study groups were balanced considering both MHC scores and baseline counts of peripheral blood CD3+ and CD4+ T lymphocytes (Table S2).

Statistical analysis using the O’Brien test
To compare untreated and IL-7-treated animals with respect to changes from baseline to multiple time points simultaneously we used the O’Brien test, which is a natural extension of the Wilcoxon rank sum test to accommodate multiple time points per subject. The analysis was used to compare simultaneously the changes from baseline to each time point during the treatment period (day 7, 14, 21, 28, and 35). First, we ranked changes from baseline to each of the time points separately, and then for each animal we averaged the ranks across the time points. To assess statistical significance, a permutation test was used. Under the null hypothesis of no effect of IL-7 treatment, one should not be able to distinguish treated monkeys from controls; the control monkeys could have been any group of 5 selected from the 11 total (excluding monkey #744 for which several time points were missing). Considering that there are 462 ways to select 5 subjects from 11, for each of these relabelings, we computed the difference between the average for animals labeled as “IL-7-treated” and the average for animals labeled as “untreated”. Afterwards, we computed the proportion of relabeled datasets that produced a mean difference at least as extreme as the actual trial data.
SUPPLEMENTARY DATA

Rapid disease progression in 4 SIV-infected macaques: re-analysis of virological and immunological data after exclusion of rapid progressors

During follow-up, two monkeys in each group (H745, H749 in the untreated group; H751, H752 in the IL-7-treated group) developed early signs of SIV-disease progression and were euthanized for humanitarian reasons after a mean of 143.5 ± 20.4 days (range, 115-163). No specific MHC allele was over- or under-represented among these 4 animals. Albeit infrequently, SIV-infected macaques have been shown to undergo a rapid disease course characterized by high-level virus replication in cells of the mononuclear phagocytic lineage rather than in CD4⁺ T cells [5]. In agreement with these previous observations, our rapid progressors (RP) showed persistently higher levels of SIV plasma viremia (Figure S1A) and antigenemia (Figure S1B), compared to conventional progressors (CP), as well as overall higher numbers of circulating CD4⁺ T cells, even though the difference reached statistical significance only on day 62 post-infection (Figure S1C). Although it is impossible, without extending the study to a larger group of animals, to establish if the RP course was affected by IL-7 treatment, the fact that two animals in each group progressed rapidly suggests that IL-7 did not influence this unique form of SIV-disease evolution.

Since the rapid disease course has been associated with an unusual pathogenesis [5], which is likely influenced by genetic factors and insensitive to IL-7 treatment, we reasoned that the inclusion of RP animals could be a confounding factor in our study, and therefore we re-analyzed all the immunological and virological data after exclusion of these 4 animals. This re-analysis showed no major changes in the statistical comparisons between treated and untreated animals regarding SIV viremia and antigenemia (Figure S2A), CD4⁺, CD8⁺, naïve, memory and effector T cells in peripheral blood (Figure S2B), ileum and lymph nodes (data not shown). However, the exclusion of RP animals had important effects on the analysis of T-cell apoptosis in lymph nodes, as described in the main text.
Changes in T-cell subpopulations in acutely SIV-infected macaques

The O’Brien permutation test was used to simultaneously compare the changes in naïve, memory and effector CD3⁺, CD4⁺ and CD8⁺ T cells from baseline to all the time points during the treatment period (day 7-35 post-infection) in IL-7-treated versus untreated animals. As shown in Figure S3A, this analysis demonstrated that the changes in the total numbers of CD3⁺, CD4⁺ and CD8⁺ T cells were significantly different in IL-7-treated and untreated macaques (Figure S3A). Likewise, significant differences between IL-7-treated and untreated animals were also observed for naïve, memory and effector CD4⁺ and CD8⁺ T cells, as well as for naïve and effector CD3⁺ T cells, but not for memory CD3⁺ T cells (Figure S3B). However, when the analysis of changes was restricted to day 14 post-infection, the differences between IL-7-treated and untreated animals were significant for all T-cell subpopulations (not shown).
SUPPLEMENTARY REFERENCES

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**SUPPLEMENTARY TABLES**

Table S1. P values for the comparison of SIV viremia, antigenemia and DNA load between IL-7-treated and untreated monkeys, obtained by Wilcoxon rank sum test. Statistically significant p values are bolded.

| Day | SIV Viremia | SIV Antigenemia | SIV DNA | Blood | GALT | Lymph nodes |
|-----|-------------|-----------------|---------|-------|------|-------------|
| 4   | 0.0430      | 0.3095          | nt      | nt    | nt   | nt          |
| 7   | 0.2403      | 0.3095          | nt      | nt    | nt   | nt          |
| 11  | 0.2403      | 0.2403          | nt      | nt    | nt   | nt          |
| 14  | 0.0823      | 0.7922          | 0.9048  | 0.4000| nt   | nt          |
| 18  | 0.9307      | 0.9307          | nt      | nt    | nt   | nt          |
| 21  | 0.7922      | 0.9307          | nt      | nt    | nt   | nt          |
| 24  | 0.4286      | 0.9307          | nt      | nt    | 0.4000| nt          |
| 28  | 0.6623      | 0.6623          | nt      | nt    | nt   | nt          |
| 35  | 0.4286      | 0.9307          | nt      | nt    | nt   | nt          |
| 41  | 0.7922      | 0.9307          | nt      | nt    | nt   | nt          |
| 48  | nt          | 0.9307          | nt      | nt    | nt   | nt          |
| 54  | nt          | 0.9307          | nt      | nt    | nt   | nt          |
| 68  | 0.6623      | 1.0000          | nt      | nt    | nt   | nt          |
| 77  | 0.7922      | 1.0000          | 0.4000  | nt    | nt   | nt          |

nt = not tested
Table S2. MHC haplotype and baseline peripheral blood CD3⁺ and CD4⁺ T-lymphocyte counts in untreated and IL-7-treated macaques.

| MHC haplotype | MHC score* | CD3⁺ T cells | CD4⁺ T cells |
|---------------|------------|--------------|--------------|
| Untreated:    |            |              |              |
| H743 A01-A08  | +2         | 1831         | 925          |
| H744 B01      | -1         | 2471         | 1774         |
| H745 A08      | +1         | 2878         | 1879         |
| H747 A08-B01  | 0          | 2097         | 1317         |
| H749 n.d.**   | 0          | 6771         | 3656         |
| H753 B08      | +1         | 3194         | 1996         |
| Mean (±SD):   | +3         | **3207±1815**| **1925±938** |

| IL-7-treated: |            |              |              |
| H746 B08      | +1         | 2604         | 1338         |
| H748 A01      | +1         | 3167         | 2211         |
| H750 A08      | +1         | 3972         | 2367         |
| H751 B01      | -1         | 3223         | 2269         |
| H752 n.d.**   | 0          | 2767         | 1486         |
| H754 A02      | 0          | 2233         | 1351         |
| Mean (±SD):   | +2         | **2994±603** | **1845±483** |

* The MHC score was calculated by attributing a positive score (+1) to alleles associated with protection from SIV disease/viral load and a negative score (-1) to alleles associated with increased viral load/accelerated disease progression; no score was attributed to alleles with no known effects on SIV disease progression.

** n.d. = none detected. The animals were negative for the MHC alleles tested (A01, A02, A08, A11, B01, B03, B04, B08, B17).
Figure S1. Levels of SIV replication and CD4⁺ T cells in conventional progressor (CP) versus rapid progressor (RP) SIV-infected macaques. Mean levels (± SEM) of SIV plasma viremia (A) and p27Gag antigenemia (B) in CP (orange) and RP (green). RP animals showed higher levels of both SIV viremia and antigenemia compared to CP at several time points, starting on day 18 post-infection, as indicated by the asterisks (*p < 0.05, ** p < 0.01, by Wilcoxon rank sum test). (C) Mean absolute numbers (± SEM) of circulating CD4⁺ T cells in CP (orange) and RP (green). A significant difference was observed on day 62 post-infection, when RP had higher mean levels of circulating CD4⁺ T cells compared to CP, as indicated by the asterisk (p < 0.042, by Wilcoxon rank sum test).
Figure S2. Reanalysis of virological and immunological parameters in IL-7-treated and untreated macaques after exclusion of macaques with rapidly progressive (RP) disease course. (A) Mean levels (± SEM) of SIV plasma viremia and p27Gag antigenemia in untreated (blue) and IL-7-treated (red) animals. (B) Mean absolute numbers (± SEM) of circulating total, naïve, memory and effector CD4⁺ and CD8⁺ T cells in untreated (blue) and IL-7-treated (red) animals. The grey shaded area indicates the IL-7-treatment period; blue and red asterisks denote significant differences with baseline values in untreated and IL-7-treated animals, respectively (*p < 0.05, ** p < 0.01, by paired Student’s t test).
Figure S3. Comparison of changes in peripheral blood T-cell subpopulations between IL-7-treated and untreated macaques during the acute phase of SIV infection. Simultaneous comparisons of changes from baseline to all the time points during acute SIV infection as analyzed by the O’Brien permutation tests. (A) Analyses for total CD3\(^+\), CD4\(^+\) and CD8\(^+\) T cells. (B) Analyses for the naïve, memory and effector T-cell subsets. The histograms represent the distribution of all possible values of the difference between IL-7-treated and untreated animals considering all the possible relabelings of the animals in the two groups (by permutation test); dots indicate the values of the differences between IL-7-treated and untreated animals for the actual trial assignments with the relative p values. Significant differences were observed in all T-cell subsets, except in memory CD3\(^+\) T cells.
Figure S4. Ki67 expression, Annexin-V binding and Bcl-2 expression in two representative animals (one untreated and one treated with IL-7). Histograms show the levels of Ki67 expression (A), Annexin-V binding (B) and Bcl-2 expression (C) at day 14 post-infection in CD4+ and CD8+ T cells from two representative animals: one from the untreated group (#749, shown in blue) and one from the IL-7-treated group (#746, shown in red).
Figure S5. SIV Gag-specific T-cell responses in IL-7-treated and untreated macaques. (A) Mean absolute numbers (± SEM) of CD4⁺ and CD8⁺ T cells producing one cytokine (single-producer, SP), two cytokines (double-producer, DP) or all three cytokines (triple-producer, TP) in response to SIV Gag peptide stimulation in untreated (shades of blue) and IL-7-treated (shades of red) animals. (B) Mean absolute numbers of SIV Gag-responding CD4⁺ and CD8⁺ T cells in untreated controls (c) and IL-7-treated animals (IL-7). The bars indicate the mean numbers of total responding cells; the colors indicate the mean numbers of IFN-γ, IL-2, or MIP-1β single-producer cells (shades of blue), IFN-γ/IL-2, IFN-γ/MIP-1β or IL-2/MIP-1β double-producer cells (shades of green), and IFN-γ/IL-2/MIP-1β triple-producer cells (purple). The numbers above each bar indicate the fraction of monkeys that gave a measurable response over background to SIV Gag peptides at the corresponding time point. The grey-shaded areas indicate the IL-7-treatment period.
Figure S6. Gating strategy used for the identification of naïve and memory T-cell subsets. Lymphocytes were selected within the lymphocyte gate in a SSC vs FSC dot plot and T cells were further selected for CD3 positivity. Within the CD3$^+$ T-cells gate, CD45RA$^-$ cells were selected and further subdivided into central memory (T$_{CM}$), transitional memory (T$_{TM}$) and effector memory (T$_{EM}$) cells by using a combination of the surface markers CCR7 and CD28 as follows: T$_{CM}$ CCR7$^+$ CD28$^+$, T$_{TM}$ CCR7$^-$ CD28$^+$ and T$_{EM}$ CCR7$^-$ CD28$^-$. Within the CD45RA$^+$ subpopulation, naïve (T$_N$) and terminal effector (T$_{TE}$) cells were identified using the same combination of CCR7 and CD28 markers as double positive and double negative cells, respectively. CD4$^+$ and CD8$^+$ T-cell lineages cells were subsequently identified on the gated subpopulations.