Involvement of IL-15 in the Pathogenesis of Human T Lymphotrophic Virus Type I-Associated Myelopathy/Tropical Spastic Paraparesis: Implications for Therapy with a Monoclonal Antibody Directed to the IL-2/15R β Receptor

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Involvement of IL-15 in the Pathogenesis of Human T Lymphotropic Virus Type I-Associated Myelopathy/Tropical Spastic Paraparesis: Implications for Therapy with a Monoclonal Antibody Directed to the IL-2/15Rβ Receptor

Nazli Azimi,* Steven Jacobson, † Thomas Leist,† and Thomas A. Waldmann*

Human T lymphotropic virus type I (HTLV-I) is the causative agent of an inflammatory neurological disease termed HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP). An ongoing lymphocyte activation exists in patients with HAM/TSP, which was demonstrated by the spontaneous proliferation of their PBMC ex vivo. It was shown that spontaneous proliferation present in HAM/TSP is due, in part, to an IL-2/IL-2R autocrine loop. However, addition of Abs against IL-2 or IL-2Rα only partially inhibited the spontaneous proliferation. Since IL-15 is a cytokine with similar functional characteristics to those of IL-2, we reasoned that IL-15 might be an additional growth factor that contributes to the spontaneous proliferation observed in HAM/TSP. In this study, we demonstrated that IL-15 mRNA expression was elevated in PBMC obtained from HAM/TSP patients when compared with those of the normal donors. Furthermore, we showed that the addition of blocking Abs against IL-15 or its receptor inhibited the spontaneous proliferation of HAM/TSP PBMC. Addition of Abs directed toward both IL-15 and IL-2, or their receptors, inhibited the proliferation almost completely. These data suggest the existence of two autocrine loops involving IL-15/IL-15R and IL-2/IL-2R, both contributing to the spontaneous proliferation of HAM/TSP PBMC. The Journal of Immunology, 1999, 163: 4064–4072.

Infection with the human T cell lymphotropic virus type I (HTLV-I) is associated with adult T cell leukemia/lymphoma (ATL) (1) and an inflammatory neurological disease termed HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (2, 3). Recent evidence has suggested that the virus is also associated with other inflammatory diseases, including uveitis, arthritis, myositis, and alveolitis (4–8). It has been proposed that the panoply of disease states associated with HTLV-I infection may result from the expression of a unique sequence called pX within the proviral genome (9). pX encodes a transcrip-
that the anti-IL-2Rb Ab blocks the interaction of IL-15 with IL-2/15Rb, prompted us to examine the potential role of the IL-15 in the ex vivo spontaneous proliferation of PBMC from HAM/TSP patients. In this study, by utilizing an RNase protection assay (RPA) and competitive RT-PCR techniques, we demonstrated that IL-15 mRNA expression is elevated in the ex vivo PBMC obtained from HAM/TSP patients when compared with that of the normal donors. IL-15 mRNA levels were increased in both T cells and non-T cell populations of the HAM/TSP PBMC when compared with those of the normal donors. This IL-15 overexpression may contribute to the spontaneous proliferation of the HAM/TSP PBMC. This hypothesis is supported by our demonstration that addition of an anti-IL-15 Ab to the ex vivo PBMC cultures inhibited the spontaneous proliferation of these cells. Furthermore, we demonstrated that the simultaneous addition of the anti-IL-2 and anti-IL-15 Abs inhibited proliferation more than either Ab alone. This observation suggests that there are two autocrine loops involving IL-15 as well as IL-2, which support the spontaneous proliferation of the HAM/TSP ex vivo PBMC. On the basis of these observations, we suggest that IL-15 overexpression in HAM/TSP PBMC contributes to the activated proliferation state of the T cells. These activated cycling T cells may contribute to the inflammatory nervous system damage in the HAM/TSP disorder. Thus, therapy directed toward IL-15 or toward the IL-2/15Rb receptor subunit may be of value in the treatment of this disease.

Materials and Methods

Proliferation assay

PBMC from HAM/TSP patients were cultured in RPMI 1640 (Life Technologies, Gaithersburg, MD), 10% human AB serum (Sigma, St. Louis, MO), 0.3 mg/ml glutamine (Life Technologies), 100 U/ml penicillin and streptomycin (Life Technologies) and incubated at 37°C and 5% CO2, 95% air. A total of 10^5 cells was incubated with media alone or with 2.5, 5, 10, or 20 μg/ml concentrations of a control Ab, UPC10, which is a nonspecific murine IgG2a Ig (Sigma), anti-IL-2 Ab (a neutralizing polyclonal anti-IL-2 Ab, a gift from Hoffmann-LaRoche, Nutley, NJ), an anti-IL-15 Ab M111 (Genzyme, Cambridge, MA), an anti-IL-2/RAb Ab, anti-Tac, (Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD) (24), or an anti-IL-2/15Rb Ab, Mikβ1 (a gift from Mitsuru Tsudo, Tokyo Metropolitan Institute of Medical Sciences, Tokyo, Japan) (25). Cells were pulsed after 6 days of culture for 6 h with 1 μCi [3H]thymidine. Each assay was performed in triplicate. The average cpm from each of the patients was plotted with SEs shown for each group.

To examine the specificity of the Abs used in this assay, a proliferation assay utilizing Kit225-K6 T cells (cytokine IL-2-, IL-7-, and IL-15-depent) was performed. Cells were seeded with exogenous IL-2 (a gift from Hoffmann-LaRoche) and IL-15 (Genzyme, Cambridge, MA), an anti-IL-2/RAb Ab, anti-Tac, (Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD) (24), or an anti-IL-2/15Rb Ab, Mikβ1 (a gift from Mitsuru Tsudo, Tokyo Metropolitan Institute of Medical Sciences, Tokyo, Japan) (25). Cells were pulsed after 6 days of culture for 6 h with 1 μCi [3H]thymidine. Each assay was performed in triplicate. The average cpm from each of the patients was plotted with SEs shown for each group.

Isolation of peripheral T cells

 Eleven HAM/TSP patients and five seronegative healthy donors who were admitted to the Clinical Center of the National Institutes of Health were studied. PBMC were separated from heparinized blood by density gradient centrifugation. T cells were isolated from PBMC employing magnetic bead separation using anti-CD3 Abs conjugated to the magnetic beads, as described in the manufacturer’s protocol (Miltenyi Biotec, Auburn, CA). T cells were eluted from the magnetic column and collected as the T cell population. The efficiency of T cell isolation was determined using FACS analysis with CD3 staining of the isolated T cells. The purity of the enriched T cell population for 11 HAM/TSP patients and 5 normal donors was >96%. The fraction of cells that passed through the CD3+ column was collected as the non-T cell population. There was <5% T cell contamination in non-T cell population as determined by FACS analysis utilizing an anti-CD3 Ab.

RPA

Total RNA was obtained from patient PBMC using PUREscript (Gentra Systems, Minneapolis, MN). Ten micrograms of total RNA were used in a RPA that was performed on patient and normal donors’ PBMC RNA samples, as described previously (20). Human IL-15, IL-2, and GAPDH (housekeeping gene) were included in a RPA probe set (PharMingen, San Diego, CA).

Competitive RT-PCR

Competitive PCR was utilized to quantitate IL-15 and IL-2 transcripts using cDNA generated from the RT reaction. The RT reaction was performed using a DNA cycle kit (Invitrogen, Carlsbad, CA) and 2 μg of total RNA, according to the manufacturer’s protocol. One microliter of each RT reaction was used to amplify β-actin cDNA using CAC CCT CTCA AAA TGA GCT GCG (sense) and CAG CAC TGT GTG GGC GTA CAG G (anti-sense) primers to normalize each RT reaction. One microliter of RT reaction from T cells was used to amplify Tax mRNA. About 2 μl of each RT reaction were used for competitive PCR. The competitor template for IL-15 was generated by inserting a 60-bp fragment (part of the IL-15 intron five sequence) in IL-15 cDNA BgIII site. Using the primers GAA ACC ACA TTT GAG AAT TTT TTC (sense) and CCA TTA GAA GAC AAA CTG TTT (anti-sense), endogenous IL-15 cDNA amplification was competed by adding a known concentration of the competitor. The amount of the competitor used for each reaction is indicated in the figure legends. Endogenously amplified IL-15 cDNA migrated below the competitor IL-15. The conditions for the PCR reaction were 95°C, 5 min (for one cycle); 95°C, 1 min; 55°C, 2 min; 72°C, 3 min (for 30 cycles); and 72°C, 5 min (for one cycle). Ten microliters of each PCR reaction were run on a 2% agarose gel.

The competitor template for the IL-2 was generated using two consecutive PCR reactions. The first PCR reaction was performed using the first primer set of CTG CTG GAT TTA CAG ATG ATT TTT (sense) and TTC AGA TCC CTT TAG TTC CAG AAC + GCT TTT AGG TAA ATT TAG CAC TTC (anti-sense) and IL-2 cDNA as template. The anti-sense primer was a chimeric primer that joined two consecutive sequences from the IL-2 cDNA at the position indicated by an asterisk. The PCR product obtained from the first reaction was used as a template for the second PCR reaction with the primer set of CTG CTG GAT TTA CAG ATG ATT TTT (sense) and TTT AGA CTT CTT TAG TTC CAG AAC (anti-sense). The final PCR product used as a competitor was an IL-2 cDNA that was missing 76 base pairs. The competitive PCR reaction was performed as described above. Endogenously amplified IL-2 cDNA migrated above the competitor IL-2. The PCR conditions were the same as those for the IL-15 amplification. The geometric means were calculated for patients and normal donor groups and were subsequently used to determine the p values. All the p values were defined in comparison to the normal donors group using the Student’s t test.

HTLV-I Tax RT-PCR

One microliter of the RT reaction performed on T cell RNA (Fig. 2) was used to amplify Tax cDNA. A nested PCR experiment was conducted using one primer set of ATC CGG TGG AGA CTC CTC AA (sense) and CGT GCC ATC GGT AAA TGT CC (anti-sense) and second primer set of ACT CCT CAA CGG AGC TGC ATG (sense) and AAA GGG TGG TGG GCA AAC AGT C (anti-sense). These primers were designed to span over an intron in the HTLV-I genome to avoid any amplification of the HTLV-I genome. The PCR conditions were the same as described above for IL-2 and IL-15 RT-PCR amplifications. Ten microliters of the PCR reaction were run on the 1% agarose gel.

Results

IL-15 mRNA expression was elevated in PBMC of the HAM/TSP patients

As part of our effort to determine whether IL-15 plays a role in the spontaneous lymphoproliferation from HAM/TSP patients, we examined IL-15 mRNA expression in the PBMC. To study IL-15 and IL-2 gene expression in the HAM/TSP patients, we examined IL-15 and IL-2 mRNA levels from unstimulated PBMC obtained ex vivo from 11 HAM/TSP patients and compared these values with IL-15 and IL-2 mRNA levels from 5 normal donors. Total RNA extracted from HAM/TSP and normal donor PBMC was used in an RPA to determine IL-15, IL-2, and GAPDH mRNA...
levels. Since we observed some loading variations between samples from patients and normal donors (Fig. 1), we conducted densitometry analysis to standardize the data. The induction index shown in this figure is compensated on the basis of GAPDH signals. As shown in Fig. 1, the IL-15 mRNA levels were elevated in HAM/TSP patients, between 1.5- and 4.2-fold over those of normal donors ($p < 0.0035$). In the same assay, using an IL-2 specific probe, no IL-2 mRNA was detected in any of the patient or normal donor samples. Since IL-2 is predominantly produced by activated T cells, it is possible that its mRNA in the total PBMC pool became too diluted by that of non-T cells to be detected by RPA. Therefore, IL-2 mRNA levels were measured in the T cells isolated from the HAM/TSP PBMC as discussed below.

**IL-15 and IL-2 mRNA levels were up-regulated in T cells isolated from HAM/TSP patients**

To study the effect of HTLV-I infection on IL-15 and IL-2 gene expression, the production of mRNA encoding these two cytokines was examined in T cells in which a subset (CD4+) is thought to be infected by HTLV-I in HAM/TSP patients. Unstimulated PBMCs obtained ex vivo from 11 HAM/TSP patients and 5 normal donors were used as the starting material for the separation of their T cells.

**FIGURE 2.** A, Competitive RT-PCR using RNA isolated from T cells enriched from 11 HAM/TSP patients or 5 normal donors PBMC (only 1 normal donor [ND] is shown) using CD3 magnetic bead separation. Two micrograms of the total RNA were used in the RT reaction to generate cDNA. About 2 μl of the generated cDNA were used in a competitive PCR using varying concentrations of the competitor template. Endogenously amplified IL-2 cDNA migrates above the competitor and endogenously amplified IL-15 cDNA migrates below the competitor in the agarose gels. Numbers 1–5 indicate the amount of the competitor DNA added to each reaction set. The quantity of the competitor added to detect IL-2 in normal donors were: 1, $10^{-3}$; 2, $5 \times 10^{-3}$; 3, $10^{-4}$; 4, $5 \times 10^{-5}$; and 5, $10^{-6}$ ng. The quantities of the competitor added to detect IL-15 were the same as those for IL-2 in both normal donors and HAM/TSP patients. Table I shows the amount of the competitor DNA in ng required to achieve equal intensity signals between the two PCR products originated from either endogenous or competitive cDNA. B, β-actin PCR amplification. One microliter of the same RT reaction performed on T cells RNA (A) was used in an PCR reaction to amplify β-actin cDNA to serve as a control to normalize for the cDNA input in IL-2 and IL-15 competitive PCR reactions.
Table I. The comparison between IL-2 and IL-15 mRNA levels from the HAM/TSP patients and those of the normal donors as determined by RT-PCR analysis

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IL-2 and IL-15 mRNA levels are indicated in this table as the dilution factor of the competitor required to achieve equal intensity signals between the two PCR products originated from either endogenous or competitive cDNA. The geometric means of the IL-15 and IL-2 mRNA levels from 5 normal donors and 11 HAM/TSP patients are included in this table. The p values comparing two groups, as indicated in Table I, were calculated for HAM/TSP patients and normal donor groups, as indicated in Table I. These values were used to determine the p value comparing two groups. As shown in Table I, the IL-2 mRNA levels were elevated in HAM/TSP T cells by ~4-fold when compared with those of normal donors (p = 0.015). The IL-15 mRNA levels were also elevated ~5-fold in HAM/TSP T cells when compared with those of the normal donors (p = 0.027).

β-actin cDNA was PCR amplified from the same RT reaction used above to serve as a control to normalize for the cDNA input in IL-2 and IL-15 competitive PCR reactions (Fig. 2B). These data suggest that T cells of HAM/TSP patients could be the source of the IL-2 and IL-15 that contributes to the spontaneous proliferation observed in this disorder.

HTLV-I Tax was produced in HAM/TSP PBMC

It has been demonstrated that the HTLV-I Tax protein transactivates a number of cellular genes, including those of IL-2, IL-2Rα, and IL-15 (10, 11, 20). To detect Tax expression in HAM/TSP patients, a sensitive RT-PCR assay was used to detect Tax mRNA. As mentioned in Materials and Methods, from the RT reaction performed on the T cells mRNA, 1 μl was used to amplify Tax cDNA in a nested PCR reaction. The β-actin PCR shown in Fig. 2B represents the cDNA input for each sample. As shown in Fig. 3, Tax mRNA was detected in the PBMC from all the HAM/TSP patients but not from five normal donors. These data support the view that Tax is expressed by HTLV-I-infected HAM/TSP patient cells and may up-regulate transcription of several host genes, including those of IL-2 and IL-15.

IL-15 mRNA levels were up-regulated in non-T cells isolated from HAM/TSP patients

Since activated monocytes and macrophages are the main sources of the IL-15 production (26), we quantitated the IL-15 mRNA expression in cell populations other than T cells in HAM/TSP patients. In the process of magnetic bead T cell isolation, two populations were collected: T cells that were eluted from the separation column and the non-T cell population that was effectively depleted from T cells by ~96%, as determined by FACS analysis (data not shown). RNA was extracted from HAM/TSP and normal donor non-T cell populations and was examined by competitive RT-PCR to quantitate IL-15 and IL-2 mRNA, as described in Materials and Methods. The range of the competitor used in IL-2 PCR reaction was wider for HAM/TSP patients (10^{-1}–10^{-7} ng) vs normal donors (10^{-3}–10^{-7} ng). The range of the competitor used in the IL-15 PCR was 10^{-2}–10^{-7} ng for normal donors and 10^{-3}–10^{-7} ng for HAM/TSP patients. Fig. 4A demonstrates the results from the PCR experiment. The data obtained from Fig. 4A are reported in Table I as the ng amount of the original competitor.

T cells were isolated using a positive selection method involving Abs directed against CD3 that were conjugated to the magnetic beads. The T cell purity following separation was determined using FACS analysis by staining for the CD3 marker on the purified population. In light of the limited amount of RNA available, the RNA isolated from these T cells was evaluated in a competitive RT-PCR assay established for IL-15 and IL-2 mRNA quantitation, as indicated in Materials and Methods. To cover extreme variations in IL-2 or IL-15 mRNA expressions in HAM/TSP patients, a wider range of the competitor was used for HAM/TSP patients (10^{-1}–10^{-7} ng) than was used for the normal donors (10^{-3}–10^{-5} ng). Fig. 2A demonstrates the results of the PCR experiment for each sample. The data obtained from Fig. 2A are reported in Table I as the ng amount of the original competitor required to achieve equal intensity signals between the two PCR products originated from either endogenous or competitive cDNA. The geometric means were calculated for HAM/TSP patients and normal donor groups, as indicated in Table I. These values were used to determine the p value comparing two groups. As shown in Table I, the IL-2 mRNA levels were elevated in HAM/TSP T cells by ~4-fold when compared with those of normal donors (p = 0.015). The IL-15 mRNA levels were also elevated ~5-fold in HAM/TSP T cells when compared with those of the normal donors (p = 0.027).

β-actin cDNA was PCR amplified from the same RT reaction used above to serve as a control to normalize for the cDNA input in IL-2 and IL-15 competitive PCR reactions (Fig. 2B). These data suggest that T cells of HAM/TSP patients could be the source of the IL-2 and IL-15 that contributes to the spontaneous proliferation observed in this disorder.
required to achieve equal intensity signals between the two PCR products originated from either endogenous or competitive cDNA. The geometric means were calculated for HAM/TSP patients and normal donor groups, as indicated in Table I. These values were used to determine the $p$ value comparing two groups. As shown in Table I, endogenous IL-2 mRNA was not detectable in the non-T cell population at a concentration as low as $10^{-7}$ ng of the competitor. This finding is in accord with previous reports that T cells are the main source of IL-2 production. In contrast, IL-15 mRNA levels were elevated and were $50$-fold higher in HAM/TSP patients than in normal donors ($p = 0.0001$). $\beta$-actin cDNA was PCR amplified from the same RT reaction used above to serve as a control to normalize for the cDNA input in IL-2 and IL-15 competitive PCR reactions (Fig. 4B). This observation suggests that both T and non-T cell populations contribute to the IL-15 generation.

**IL-15 mRNA was up-regulated in non-lymphoid cells by HTLV-I Tax protein**

The fact that IL-15 mRNA level is over-expressed in the non-T cell population of HAM/TSP PBMC raises the question of which element(s) up-regulates IL-15 transcription in these cells. One possibility is that HTLV-I-infected T cells secrete cytokines such as TNF-$\alpha$ and IFN-$\gamma$ or the virally encoded Tax, which in turn could...

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**FIGURE 3.** An RT-PCR analysis of the HTLV-I Tax was performed on T cells isolated from normal donors and HAM/TSP PBMC using CD3 magnetic bead separation. One microliter of RT reaction performed on the RNA obtained from T cell population (same RT reaction used in Fig. 2) was used to amplify Tax cDNA. Tax mRNA expression was measured in all the HAM/TSP patients PBMC using RT-PCR (nested PCR), but not in normal donor PBL. Since the same RT reaction in Fig. 2 was used as the template for Tax PCR, the $\beta$-actin shown in Fig. 2B serves as a control for Tax PCR.

**FIGURE 4.** A, Competitive RT-PCR using RNA isolated from non-T cells isolated from 11 HAM/TSP patients or 5 normal donors PBMC (only 1 normal donor is shown in Fig. 4) by CD3 magnetic bead separation. The population of PBMC that was not selected by the CD3 beads but passed through the column was termed the non-T cell population. Two micrograms of the total RNA isolated from this latter population were used in a RT reaction to generate cDNA. Almost 2 $\mu$L obtained by this RT reaction were used in a competitive PCR using varying concentrations of the competitor template. Endogenously amplified IL-2 cDNA migrates below the competitor and endogenously amplified IL-15 cDNA migrates above the competitor in an agarose gel. Numbers 1–5 indicate the amount of the competitor DNA added to each reaction set. The quantity of the competitor added to detect IL-2 in normal donors were: 1, $10^{-2}$; 2, $10^{-3}$; 3, $10^{-4}$; 4, $10^{-5}$; and 5, $10^{-7}$ and for HAM/TSP patients were: 1, $10^{-2}$; 2, $10^{-3}$; 3, $10^{-4}$; 4, $10^{-5}$; and 5, $10^{-7}$ ng. Table I shows the amount of the competitor DNA in ng required to achieve equal intensity signals between the two PCR products originated from either endogenous or competitive cDNA. B, $\beta$-actin PCR amplification. One microliter of the same RT reaction performed on the non-T cell RNA (A) was used in an PCR reaction to amplify $\beta$-actin cDNA to serve as a control to normalize for the cDNA input in IL-2 and IL-15 competitive PCR reactions.
up-regulate IL-15 gene expression in neighboring cells (20, 27). Another possibility is that nonlymphoid cells may become infected with HTLV-I. There is evidence suggesting that astrocytes from HAM/TSP brain contain the HTLV-I Tax sequence (28). To examine the impact of HTLV-I infection on IL-15 expression in non-lymphoid cells, we examined IL-15 mRNA production in two astrocyte cell lines stably transfected with an HTLV-I Tax construct. As shown in Fig. 5A, using an RPA procedure, the IL-15 mRNA level was shown to be increased 2.1- and 3.6-fold, as determined by phosphoimaging densitometry in the Tax transfectant cell lines 2PC2 and 2PD2, respectively, when compared with that of the parental cell line U251. To determine whether there is an up-regulation by HTLV-I Tax of the IL-15 mRNA expression in another nonlymphoid cell line, we transiently transfected two monocytic cell lines, SAML and KG1, with a Tax construct (20). IL-15 mRNA expression was examined using an RPA. As shown in Fig. 5B, IL-15 mRNA level was increased from a nondetectable to a detectable level. To determine whether transfection of the Tax construct occurred successfully in these cells, an RT-PCR experiment was performed. As shown in Fig. 5C, Tax cDNA was amplified only in cells after transfection of the Tax construct.

FIGURE 5. A, RPA performed on the RNA isolated from two astrocytic cell lines, 2PC2 and 2PD2, transfected stably with a Tax construct and their parental cell line U251 using IL-15 and GAPDH probes. The intensity of the IL-15 band was determined to be 2.1-fold with 2PC2 and 3.6-fold with 2PD2 greater than that of the U251, as determined by phosphoimager densitometry. B, IL-15 mRNA was detected by RPA in two monocytic cell lines, KG1 and SAML, only after transient transfection of a Tax construct into these cells. C, Expression of Tax plasmid was confirmed by conducting an RT-PCR experiment on KG1 and SAML cells before and after HTLV-I Tax construct transfection. Tax cDNA was amplified only in cells after transfection of the Tax construct.

FIGURE 6. The spontaneous proliferation of PBMC from 11 HAM/TSP patients in the presence of Abs against IL-2, IL-15, IL-2Rα (anti-Tac), and IL-2R/15β (MiKβ1) at concentrations of 2.5, 5, 10, and 20 μg/ml. The proliferation is shown as total cpm after the cells were pulsed with [3H]thymidine. For each experiment, 10^5 PBL cells were used per well in a 96-well plate. Medium only (no Ab), UPC10 (control Ab), anti-IL-2, anti-IL-15, anti-IL-2 plus anti-IL-15, anti-Tac, MiKβ1, or anti-Tac plus MiKβ1 were added to each well to yield a total volume of 200 μl per well. Cells were incubated for 6 days and then were pulsed with 1 μCi of the [3H]thymidine. Cells were harvested after 6 h. Each experiment was performed in triplicate, and the SEM was calculated for each time point. As shown in this graph, both anti-IL-2 and anti-IL-15 Abs partially inhibited the spontaneous proliferation in a dose-dependent manner. Combination of both of these Abs increased their impact in blocking the PBMC proliferation. Anti-Tac and MiKβ1, Abs to the cytokine receptors, also inhibited the PBMC proliferation. Their combination almost completely blocked the PBMC proliferation.
IL-15 contributed to the spontaneous proliferation of PBMC from patients with HAM/TSP

Up to this point, we demonstrated that IL-15 mRNA was over-expressed in HAM/TSP PBMC compared with that of the normal donors. To examine any potential influence that IL-15 over-expression might have on the HAM/TSP spontaneous proliferation, we studied the PBMC of the HAM/TSP patients in spontaneous proliferation assays, as described in Materials and Methods. The proliferation of PBMC in ex vivo culture was measured by quantitating the thymidine incorporation in the absence and presence of Abs blocking IL-2, IL-15, or their receptors. The fidelity of these Abs was examined using an IL-2- and IL-15-dependent Kit225 T cell line. As shown in Fig. 6, when a neutralizing Ab against the IL-2 cytokine was added to the PBMC of the HAM/TSP patients, the proliferation was inhibited by ~45–75% at each Ab concentration compared with the proliferation in the presence of media alone or a control Ab (UPC10). Similarly, the addition of the neutralizing Ab against IL-15 (M111) inhibited 40–50% of the proliferation observed with control Ab or media alone. Furthermore, the simultaneous addition of anti-IL-2 and anti-IL-15 Abs, blocked the proliferation almost completely (~80–95% of the proliferation observed with control Ab or media alone). The difference between the use of both Abs vs each Ab alone was significant (the p value for IL-2 vs both Abs was 0.0547 and for IL-15 vs both Abs was 0.0218). This suggests that both IL-15 and IL-2 contribute and have an additive effect on the HAM/TSP PBMC proliferation.

To examine the involvement of IL-2 and IL-15 receptors in the proposed autocrine loop, we studied the effect of the addition of blocking Abs against IL-2Rα and IL-2Rβ. Addition of the anti-Tac Ab (anti-IL-2Rα Ab) inhibited the PBMC proliferation by ~82–87%, presumably by interfering with the interaction of IL-2 with IL-2R. Similarly, MiKβ1 (anti-IL-2/15Rβ Ab) inhibited the proliferation by 70–78%, compared with the proliferation of the control Ab or media alone. However, this blockade was presumably not through the inhibition of the interaction of IL-2 with IL-2Rβ since we and others have reported that MiKβ1 does not block the IL-2-mediated functions on cells expressing the high affinity form of IL-2 receptor-expressing α-, β-, and γ-chains (21). In contrast, MiKβ1 Ab does block the interaction of the IL-15 with IL-2/15Rβ (20, 21). The fact that the addition of the combined anti-Tac and MiKβ1 Abs blocked PBMC proliferation almost completely (>90%) supports the hypothesis that these two Abs block two separate autocrine loops. These data suggest that IL-15, in addition to IL-2, contributes to the spontaneous proliferation of the HAM/TSP PBMC. Taken as a whole, the data support two Tax-induced autocrine loops, one involving IL-2 and the other involving IL-15 that together drive the spontaneous proliferation of the PBMC of patients with HAM/TSP.

Discussion

In this study, we demonstrated that IL-15 mRNA was up-regulated in HAM/TSP PBMC when compared with that of the normal donors. Since CD4+ T cells are known to be the reservoir of HTLV-I (29), it is reasonable to suggest that up-regulation of IL-15 mRNA in the T cells is due to HTLV-I infection and Tax expression. This Tax protein has been shown to induce the transcription of many cellular genes, including that of IL-15 (20). This view is supported by the observation in this study that HTLV-I Tax mRNA was expressed in all the HAM/TSP patients. However, we were not able to establish a correlation between the level of HTLV-I Tax expression and IL-15 or IL-2 mRNA levels in the HAM/TSP patients.

As demonstrated in this study, IL-15 mRNA levels were also elevated in both T and non-T cell populations of the HAM/TSP PBMCs when compared with those of the normal donors. The mechanism of IL-15 up-regulation in T cells may be the HTLV-I infection of CD4+ cells. Up-regulation of IL-15 in non-T cells, in contrast, may be due to the HTLV-I infection of nonlymphoid cells. There are reports indicating that dendritic cells (30, 31), epithelial cells (32), fibroblasts (33), and astrocytes (28, 34) from HAM/TSP patients can be infected with HTLV-I. Therefore, it is possible that cells other than T cells became infected by the virus, which may explain the up-regulation of the IL-15 gene in non-T cell populations. We showed in this study that HTLV-I Tax transfection into astrocytes and mononuclear cells up-regulated IL-15 expression in vitro.

Another possible mechanism for IL-15 up-regulation in non-T cells is suggested by reports indicating that IL-15 production is up-regulated by addition of factors such as LPS and IFN-γ (35–37). These factors are known to be strong inducers of transcriptional factors that regulate IL-15 transcription, including NF-κB and IFN regulatory factor-1 (IRF-1) (38, 39). There are reports indicating that in HAM/TSP there is an up-regulation of cytokines such as IFN-γ and TNF-α (27, 40–42), all strong inducers of NF-κB or IRF-1 transcription factors. Thus, it is possible that up-regulation of IL-15 in non-T cells is an event secondary to the HTLV-I infection of the T cells. Infected T cells may produce cytokines including IFN-γ and TNF-α, which in turn act on circulating macrophages and monocytes, resulting in expression of IL-15 by these cells. The magnitude of IL-15 overexpression in these cells is higher than that of T cells, reflecting the fact that monocytes and macrophages are normally the main sources of IL-15 production in physiological conditions and, therefore, are more responsive to stimuli up-regulating IL-15 gene expression (26).

However, a more thorough study is required to study IL-15 gene expression in macrophages and monocytes in HAM/TSP patients vs normal donors by positively selecting these cells with magnetic beads and studying them as described here.

We previously showed that HAM/TSP PBMC spontaneous proliferation could be blocked in part by the addition of Abs directed against IL-2 or its receptor, IL-2Rα, by blocking the IL-2/IL-2Rα autocrine loop (19). In this study, we demonstrated that HAM/TSP PBMC spontaneous proliferation could also be inhibited using Abs against IL-15 or its receptor, IL-2/15Rβ. This observation suggests the presence of an IL-15/IL-15R autocrine loop similar to that of the IL-2/IL-2R. It is also interesting to note that the Abs against receptors impact proliferation more profoundly than the Abs against cytokines themselves. It is possible that an anti-Receptor Ab disturbs the stability of the cell surface (43) and, therefore, exerts a more effective inhibition on proliferation. The fact that the simultaneous addition of Abs against both IL-2 and IL-15 or their receptors inhibits the proliferation almost completely indicates that the IL-2/IL-2R and IL-15/IL-15R autocrine loops complement each other, both contributing to the HAM/TSP PBMC spontaneous proliferation.

IL-15 overexpression in HAM/TSP patients may have implications for understanding the pathogenesis of this disease and other CNS inflammatory diseases, such as multiple sclerosis. There are a variety of physiological responses and inflammatory reactions that are associated with IL-2 in the CNS (44). However, IL-2 expression in the CNS is apparently very low and regionally restricted (44). Considering the functional similarities between IL-2 and IL-15, it is reasonable to search for an intrinsic source of IL-15 production in the CNS. IL-15 and its receptors, including the IL-15Rα, β-, and γ-chains have been demonstrated throughout the
mouse CNS, and IL-15 has been shown to be produced by microglial cells, which are responsible for secretion of many CNS cytokines (34, 45). The IL-15/IL-15 receptor system was shown to be functional in microglial cells, since JAK1, one of the signaling components of the IL-15/IL-15 receptor, was found in these cells and was shown to be phosphorylated upon addition of IL-15 (34, 45). On the basis of these observations, it has been suggested that IL-15 is an intrinsically produced cytokine in the CNS that may contribute to the pathological processes in inflammatory neurological disorders by initiating a cascade of immune events that include the induction of TNF-α, IL-1β, and inflammatory chemokines. Furthermore, IL-15 has been shown to serve as a chemotaxant for T cells and an activator of CD4+ and CD8+ T cells, as well as γδ T cells (46–49). It is interesting to note that IL-15 selectively stimulates memory-type CD8+ T cells (50). The over-production of the IL-15 in the CNS may select and stimulate the proliferation of the immunopathogenic CD8+ T cells. In addition, IL-15 is known to be an indispensable factor for the growth and differentiation of NK cells (37, 51). The expression of the IL-15 cytokine by virally infected cells has been shown to stimulate NK cell activity (52).

Furthermore, IL-15 has been shown to serve as a chemoattractant for T cells and to contribute to the pathological processes in inflammatory neurological disorders by initiating a cascade of immune events that include the induction of TNF-α, IL-1β, and inflammatory chemokines. Furthermore, IL-15 has been shown to serve as a chemotaxant for T cells and an activator of CD4+ and CD8+ T cells, as well as γδ T cells (46–49). It is interesting to note that IL-15 selectively stimulates memory-type CD8+ T cells (50). The over-production of the IL-15 in the CNS may select and stimulate the proliferation of the immunopathogenic CD8+ T cells. In addition, IL-15 is known to be an indispensable factor for the growth and differentiation of NK cells (37, 51). The expression of the IL-15 cytokine by virally infected cells has been shown to stimulate NK cell activity (52).

In summary, we have demonstrated that IL-15 mRNA is up-regulated in HTLV-I Tax-expressing PBMC of patients with HAM/TSP. Furthermore, the spontaneous PBMC proliferation observed was inhibited by the simultaneous addition of Abs to IL-15 and IL-2 or by Abs directed toward the receptors for these cytokines. These observations have implications for the therapy of patients with HAM/TSP, and potentially multiple sclerosis as well. We have recently completed a trial of humanized anti-Tac (Zenapax) directed against IL-2Rα, that is specific for IL-2, in patients with HAM/TSP (55). The response was encouraging with a reduction in the viral load and the spontaneous proliferation of PBMC ex vivo. Our present study provides the scientific basis for the addition of an Ab that blocks the IL-15 action (for example, humanized Mklβ1Ab, directed toward IL-2/15β) in such therapeutic trials. Thus, our emerging understanding of the roles of IL-2 and IL-15 and their receptors in the pathogenesis of the immunological abnormalities of HAM/TSP suggests novel approaches to the therapy of these patients.

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References