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Serotonin regulation of T-cell subpopulations and of macrophage accessory function

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SUMMARY

The role of serotonin as an immune modulator was investigated by measuring the functional competence of T cells from control mice versus from mice whose intracellular stores of serotonin had been depleted by pretreatment with p-chlorophenylalanine (PCPA). While the proportions of splenic $CD4^+$ and $CD8^+$ T cells isolated from control and PCPA-treated mice were similar, the level of expression of the α-chain interleukin-2 receptor (IL-2R) was reduced on splenic $CD4^+$ cells but not on $CD8^+$ cells. Culture with the T-cell mitogen concanavalin A (Con A) failed to induce expression of the IL-2R on either $CD4^+$ or $CD8^+$ cells of PCPA-treated mice, although IL-2R was induced on control cells. The proliferative response to Con A by these spleen cells from PCPA-treated mice was also reduced compared to that by control spleen cells. Both expression of IL-2R and proliferation in response to Con A by spleen cells from serotonin-depleted mice were increased or completely restored by supplementation of the cultures with serotonin. Studies to identify the mechanisms for the reduction in T-cell activation when serotonin levels were reduced implicated a defect in the capacity of macrophages from PCPA-treated mice to provide accessory help for T-cell activation. Splenic macrophages from control mice were able to restore the blastogenic capability of lymphocytes from PCPA-treated mice, although macrophages from PCPA-treated mice were unable to support normal lymphocyte blastogenesis unless the cultures were supplemented with serotonin. These results show the requirement of autologous serotonin for optimal T-cell activation and suggest the importance of serotonin in macrophage accessory function for T-cell activation.

INTRODUCTION

The possibility that neurotransmitters can function to regulate immune functions has been gaining increasing support. Among such neurotransmitters is serotonin (5-hydroxytryptamine; 5-HT). Besides being a neurotransmitter in the central nervous system, serotonin is also a vasoactive amine and immune modulator in the periphery. Cutaneous injection of serotonin can initiate a delayed-type hypersensitivity reaction through local recruitment and activation of $CD4^+$ T-helper cells.[1,2] Pharmacological evaluation of serotonin availability *in vivo* results in a sex- and age-selective enhancement of T-cell blastogenesis, lymphokine-activated killer cell activity and B-cell proliferation.[3] Serotonin can also directly enhance natural killer (NK) cell-mediated activity by diminishing the suppressive activities of macrophages.[4]

The immune regulatory activities of serotonin are mediated through several different 5-HT receptors. Activation of human T cells has been shown to result in increased expression of the 5-HT_{1A} receptor, which resembles the 5-HT_{1A} receptor found in the brain.[5] In addition, enhancement of murine T-cell blastogenesis by serotonin has been shown to occur through 5-HT_2 receptors.[6] In contrast to these immune stimulatory effects of serotonin, it can also inhibit some immune functions. For example, synthesis of tumour necrosis factor-α by macrophages can be inhibited with serotonin through the 5-HT_2 receptor family.[7]

Our previous studies have shown that serotonin can regulate T-cell functions in a biphasic manner, with low levels being stimulatory to T cells and high levels being suppressive.[6,8] Addition of low levels of serotonin to murine spleen cell cultures increases proliferation in response to the T-cell mitogen concanavalin A (Con A) and to interleukin-2 (IL-2). Reducing endogenous serotonin stores of mice by blocking serotonin synthesis with p-chlorophenylalanine (PCPA) results in a reduced level of T-cell blastogenesis and a reduced capacity of T cells to express the high-affinity IL-2 receptors.[6] Recent studies by others showing that PCPA treatment of activated human T-cell blasts reduces their proliferative capacity in response to IL-2 provide additional

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Abbreviations: Con A, concanavalin A; IL-2R, α chain of the interleukin-2 receptor; PCPA, p-chlorophenylalanine.

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evidence for the requirement of serotonin for optimal T-cell functions.[9] The present study aimed to identify whether $CD4^+$ versus $CD8^+$ T-cell subpopulations might be differentially affected by reduced serotonin levels, and aimed to elucidate further the mechanisms through which serotonin depletion results in diminished T-cell activation.

MATERIALS AND METHODS

Mice and treatments

The mice that were used for these studies were 6–8-week-old female C57Bl/6 mice that were obtained from Jackson Labs (Bar Harbor, ME) and then housed at the Hines VA animal research facility. To block serotonin synthesis and to deplete serotonin stores, mice were treated by intraperitoneal (i.p.) injection of 250 mg/kg of PCPA.[10] Spleen cells from these or placebo-treated mice were used 48 hr later.

Activation of T lymphocytes

Assays to measure the activation capacity of splenic T lymphocytes from control or PCPA-treated mice were performed in RPMI-1640 culture medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 20 mM HEPES buffer solution, 50 μM 2-mercaptoethanol, 2 mM L-glutamine and 10% low-endotoxin fetal bovine serum. Assessments of T-cell activation included measurement of the blastogenic response of spleen cells to optimal doses of the T-cell mitogen Con A. This was accomplished by incubating spleen cells (2×10^5) from control or PCPA-treated mice at 37° in flat-bottomed wells of microtitre plates with 4 μg/ml Con A plus various concentrations of serotonin. Some cultures were composed of Con A plus mixtures at different ratios of spleen cell fractions enriched for lymphocytes or splenic macrophages. To prepare these cell fractions, spleen cells from control or PCPA-treated mice were incubated for 1 hr, and the non-adherent cells were separated from the adherent population. The non-adherent fraction was then treated for 1 hr with 5 mM L-leucine methyl ester (LME) at 37°, as previously described,[11] to deplete monocytic cells, and was then used as the lymphocyte fraction. The adherent cells were vigorously washed to remove contaminating non-adherent or weakly adhering cells, detached with a Teflon scraper, and used as the macrophage population.

The activity of splenic T cells was also measured by their capacity to become stimulated to express the α-chain (p55) IL-2 receptor (IL-2R). Spleen cells from control or PCPA-treated mice were bulk cultured with the optimal dose of 4 μg/ml Con A for 4 days. The proportion of $CD4^+$ or $CD8^+$ cells in these cultures that also expressed the α IL-2R was determined by dual immunofluorescent staining with phycoerythrin-labelled CD4 or CD8 antibodies, and fluorescein-labelled CD25 antibodies (PharMingen, San Diego, CA), and flow cytometric analysis using a FACS 420 (Becton Dickinson, Sunnyvale, CA).

Analysis of data

The Student's *t*-test was used to determine the significance of the differences between control and experimental values. Results of Con A blastogenesis analyses are expressed as means of triplicates ± SD. All assays were repeated a minimum of three times.

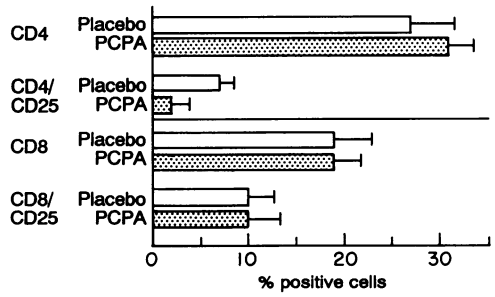

Figure 1. Reduced expression of the α-chain IL-2 receptor on freshly isolated $CD4^+$ cells from PCPA-treated mice. Freshly isolated spleen cells from control and PCPA-treated mice were immunostained with FITC-labelled antibody for the α-chain IL-2R (CD25) and with phycoerythrin-conjugated CD4 or CD8 antibody. The percentage of positively staining cells was measured by flow cytometry. Shown are means ± SD of three experiments.

RESULTS

Reduced expression of α-chain IL-2R on spleen cells of PCPA-treated mice

Studies first aimed to identify whether T-cell subpopulations might be differentially affected by a reduction in serotonin levels. Endogenous stores of serotonin were diminished by treatment of mice with PCPA (250 mg/kg), which we had previously shown to reduce splenic serotonin levels by almost half.[6] The effect of serotonin level reduction on the frequency of $CD4^+$ and $CD8^+$ cells and their expression of α-chain IL-2R (CD25) was determined. As shown in Fig. 1, freshly isolated spleen cells from control and PCPA-treated mice contained similar proportions of CD4 and CD8 cells. There were, however, differences in the proportion of these cells that also expressed the α-chain IL-2R. Twenty six per cent of the splenic CD4 cells of normal mice expressed IL-2R, while the proportion of CD4 cells from PCPA-treated mice expressing IL-2R was reduced to 6% ($P < 0.01$). In contrast, the proportion of splenic CD8 cells that co-expressed IL-2R were similar (53%) for control and PCPA-treated mice.

Also measured was the effect of serotonin depletion on the capacity of $CD4^+$ versus $CD8^+$ cells to be induced to express IL-2R after 3 days of incubation with 4 μg/ml Con A (Fig. 2). First observed was that the proportion of $CD4^+$ to $CD8^+$ cells in these cultures was similar for the control versus PCPA-treated mice. Addition of various doses of serotonin to these cells at the start of culture did not alter the proportion of $CD4^+$ and $CD8^+$ cells, regardless of whether the spleen cells were obtained from control mice or from mice whose serotonin levels had been reduced. However, reducing serotonin levels disabled the $CD4^+$ and $CD8^+$ cells from becoming stimulated to express IL-2R. Results of three separate experiments showed that after 3 days of culture with Con A, an average of 59% of the $CD4^+$ cells from control mice expressed IL-2R, while only 19% of the $CD4^+$ cells from PCPA-treated mice were induced to express IL-2R ($P < 0.01$). IL-2R expression on cultured $CD8^+$ cells from PCPA-treated mice was also less than that for control cells ($P < 0.05$), although this reduction was not as prominent as with $CD4^+$ cells. Specifically, IL-2R was expressed on 62% of the cultured $CD8^+$ cells from control

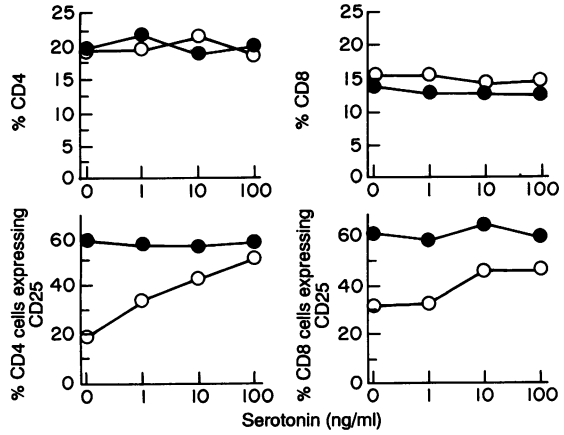

Figure 2. Requirement for serotonin for induction of IL-2R expression on both $CD4^+$ and $CD8^+$ cells. Spleen cells from control or PCPA-treated mice were cultured with 4 μg/ml Con A plus either diluent or various concentrations of serotonin. After 4 days, the frequency of $CD4^+$ and $CD8^+$ cells, and their expression of IL-2R, was measured by flow cytometry after immunostaining. Data shown are the averaged percentages (from three experiments) of $CD4^+$ and $CD8^+$ cells, and the proportions of each of these subpopulations that stained positively for IL-2R. (●) Placebo; (○) PCPA.

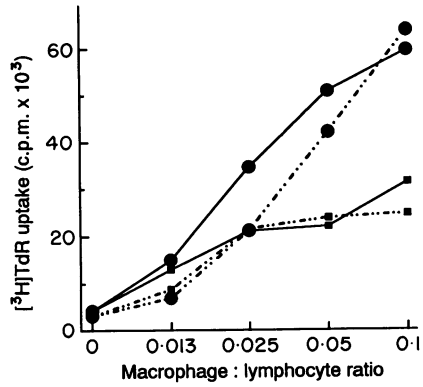

Figure 4. Inhibitory effect of serotonin depletion on macrophage accessory function for T-cell blastogenesis. Spleen cells from control and PCPA-treated mice were subdivided into adherent macrophages and non-adherent lymphocyte fractions. The lymphocytes were then admixed with increasing proportions of macrophages plus 4 μg/ml Con A. Data shown are mean c.p.m. ± SD of triplicates. (●—●) Normal macrophages plus normal lymphocytes; (●- · · -●) normal macrophages plus PCPA lymphocytes; (■—■) PCPA macrophages plus normal lymphocytes; (■- · · -■) PCPA macrophages plus PCPA lymphocytes.

mice, while only 32% of the $CD8^+$ cells from PCPA-treated mice were induced to express IL-2R. Supplementation of the cultures with increasing concentrations of serotonin resulted in a dose-dependent restoration of $CD4^+$ cell expression of IL-2R. IL-2R expression on $CD8^+$ cells was also enhanced by the addition of serotonin, although it was not completely restored, even by the highest dose of 100 ng/ml serotonin. These results indicate that the functional competence of both $CD4^+$ and $CD8^+$ cells is diminished when serotonin is reduced, and that the deficiency of $CD4^+$ cells and, to a large extent, of $CD8^+$ cells can be overcome with serotonin addition.

Dependence on serotonin for optimal T-cell proliferation to Con A

Our prior studies had suggested a requirement for serotonin in order to stimulate an optimal T-cell proliferative response to Con A.[6] This is demonstrated in Fig. 3, which compares the T-cell blastogenic response of spleen cells from control versus

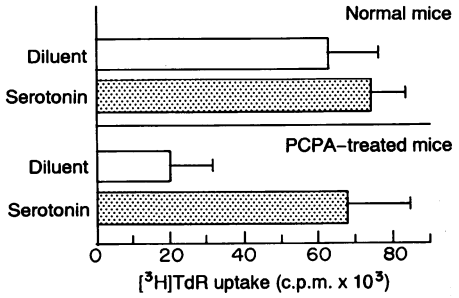

Figure 3. Requirement for serotonin for T-cell proliferative response to Con A. Spleen cells from mice that were treated with placebo or PCPA 48 hr previously were cultured with 4 μg/ml Con A plus either diluent or 100 ng/ml serotonin. Data shown are mean c.p.m. ± SD of triplicates.

PCPA-treated mice. The T-cell blastogenic response of spleen cells of PCPA-treated mice was reduced by almost 70%, compared to the splenic T-cell proliferative response of control mice ($P < 0.01$). The reduction in T-cell activation was the result of serotonin insufficiency, since addition of 100 ng/ml serotonin to the spleen cells from PCPA-treated mice restored their T-cell blastogenic responsiveness.

Effect of serotonin on the accessory function of macrophages

T-cell blastogenesis to Con A, which was shown above to require serotonin, is dependent on macrophage presence.[12] Therefore, studies determined whether the defect in T-cell blastogenesis by spleen cells from PCPA-treated mice was due to altered macrophage or altered T-cell function. This was accomplished by assessing if control splenic macrophages could provide the accessory help needed for T-cell blastogenesis to splenic lymphocytes from PCPA-treated mice, or whether lymphocytes from PCPA-treated mice remained defective in their T-cell proliferative response despite the presence of normal splenic macrophages. As shown in Fig. 4, the proliferative responsiveness of normal splenic T cells to Con A was inhibited when macrophages were depleted by adherence plus LME treatment, and was restored by replenishment with normal splenic macrophages. Adherent splenic macrophages from PCPA-treated mice were only able to increase the T-cell blastogenic response of lymphoid cells from PCPA-treated mice to the same minimal level as observed in Fig. 3 for unfractionated spleen cells from PCPA-treated mice. However, these lymphoid cells from PCPA-treated mice were not incapable of blastogenesis since adherent macrophages from control mice were able to provide the accessory help needed for their proliferative response. In the presence of macrophages from control mice, the T-cell blastogenic capacities of lymphocytes from control mice and from PCPA-treated mice were similar. In contrast, adherent splenic macrophages from

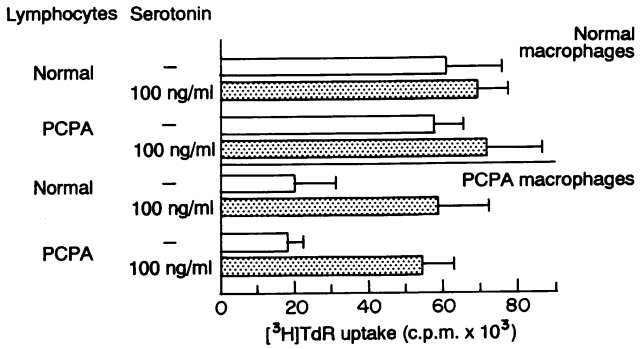

Figure 5. Serotonin restores accessory function of macrophages from PCPA-treated mice for T-cell blastogenesis. Spleen cells from control and PCPA-treated mice were subdivided into adherent macrophages and non-adherent lymphocyte fractions. The lymphocytes were then admixed with macrophages at a 10 : 1 ratio and incubated with 4 μg/ml Con A plus either diluent or 100 ng/ml serotonin. Data shown are mean c.p.m. ± SD of triplicates.

PCPA-treated mice were unable to provide this level of accessory help, leaving the T-cell response of splenic lymphoid cells from control and from PCPA-treated mice at the same low level as was observed for unfractionated spleen cells from PCPA-treated mice.

The above studies showing that macrophages from PCPA-treated mice had a reduced capacity to provide accessory help for T-cell blastogenesis, prompted studies to determine whether exogenous serotonin could restore the accessory function of macrophages from mice whose serotonin production was reduced. As was shown above, only a minimal T-cell blastogenic response occurred when macrophages from PCPA-treated mice were added to lymphocytes from either control or PCPA-treated mice (Fig. 5). However, supplementing these cultures containing macrophages from PCPA-treated mice with serotonin (100 ng/ml) completely restored the T-cell blastogenic response of non-adherent lymphoid cells from both control and PCPA-treated mice. These cultures nevertheless required macrophages, since adding serotonin (100 ng/ml) to control cultures containing only the non-adherent lymphocytes in the absence of macrophages did not result in a T-cell proliferative response (data not shown). The above results show (1) that macrophages from PCPA-treated mice are defective in their ability to provide accessory help to T cells and (2) that this deficiency is due to an insufficiency of serotonin.

DISCUSSION

That serotonin can function as a modulator of immune functions has become accepted. This modulation has been shown to include both stimulatory and inhibitory effects on T-cell, NK and macrophage activities.[1–4] The present studies reinforced our prior demonstration that optimal stimulation of T-cell function is dependent on the presence of serotonin.[6] In addition, these studies suggested that the activation potential of both the $CD4^+$ T-helper subpopulation and the $CD8^+$ suppressor/cytotoxic subpopulation is diminished when serotonin levels are reduced, although the effect of $CD4^+$ cells may be more prominent. This demonstration that serotonin has a prominent role in $CD4^+$ cell activation is consistent with a recent report that serotonin is stimulatory to the cytokine-secreting subpopulation of T cells.[9] It is also consistent with earlier reports showing that serotonin can initiate delayed-type hypersensitivity reactions either intracutaneously or within an inoculated tumour by stimulating $CD4^+$ cytokine-secreting lymphocytes which, in turn, results in an elicitation of macrophages.[1,13]

Studies performed to identify whether the defect in T-cell blastogenesis by spleen cells of serotonin-depleted mice was due to altered functions of T cells or macrophages, implicated macrophages. This implication resulted from the demonstration that the blastogenic capability of lymphocytes from PCPA-treated mice could be restored by the presence of macrophages from control mice, but not by macrophages from PCPA-treated mice. The inability of macrophages from PCPA-treated mice to provide accessory help was due to a deficiency in serotonin since lymphocytes could generate a blastogenic response in the presence of PCPA-treated macrophages when exogenous serotonin was added to the cocultures. The mechanism by which serotonin facilitates macrophage accessory function is currently unclear. One possibility is that macrophages are directly dependent on serotonin for their accessory function. Another possibility is that the absence of serotonin may result in suppressive activities of macrophages which might be moderated by the presence of serotonin. There is support for this latter possibility in earlier studies suggesting that monocytes down-regulate the functions of NK cells, but that this inhibitory effect is ameliorated by the addition of serotonin.[4,14] At present, however, there is no direct evidence to support either possibility as an explanation for the requirement for serotonin in macrophage-dependent T-cell activation.

The regulatory role of serotonin on T-cell function is not restricted to stimulating only proliferation in response to mitogens, but also includes mitogen stimulation to express α-chain IL-2R. Not fully defined is whether serotonin is also required for activating T cells in response to macrophage-independent stimuli, although this has been suggested recently.[9] Our prior studies have also supported this possibility, by showing that exogenously added serotonin enhances the proliferative responses of T cells to IL-2. However, we have not yet determined whether depletion of serotonin abrogates the capacity of T cells to respond to IL-2. The studies described in the present report nevertheless provide additional support for the importance of serotonin within the cascade that regulates T-cell functions, and have dissected some of the processes by which alterations in the serotonergic system can have profound effects on the immune system.

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REFERENCES

1. Ptak W., Geba G.P. & Askenase P.W. (1991) Initiation of delayed-type hypersensitivity by low doses of monoclonal IgE antibody. Mediation by serotonin and inhibition of histamine. *J Immunol* **146**, 3929.

2. Ptak W., Herzog W.R. & Askenase P.W. (1991) Delayed-type hypersensitivity initiation by early-acting cells that are antigen mismatched or MHC incompatible with late-acting, delayed-type hypersensitivity effector T cells. *J Immunol* **146,** 469.
3. Clancy J., Petrovic L.M., Gordon B.H., Handra R.J., Campbell D.B. & Lorens S.A. (1991) Effects of subchronic d-fenfluramine on splenic immune functions in young and old male and female Fischer 344 rats. *Int J Immunopharmacol* **13,** 1203.
4. Hellstrand K., Czerkinsky C., Ricksten A. *et al.* (1993) Role of serotonin in the regulation of interferon-gamma production by human natural killer cells. *J Interferon Res* **13,** 33.
5. Aune T.M., McGrath K.M., Sarr T., Bombara M.P. & Kelley K.A. (1993) Expression of $5HT_{1A}$ receptors on activated human T cells. *J Immunol* **151,** 1175.
6. Young M.R., Kut J.L., Coogan M.P., Wright M.A., Young M.E. & Matthews J. (1993) Stimulation of splenic T-lymphocyte function by endogenous serotonin and by low dose exogenous serotonin. *Immunology* **80,** 395.
7. Arzt E., Costas M., Finkielman S. & Nahmod V.E. (1991) Serotonin inhibition of tumor necrosis factor-α synthesis by human monocytes. *Life Sci* **48,** 2557.
8. Kut J.L., Young M.R.I., Crayton J.W., Wright M.A. & Young M.E. (1992) Regulation of murine T-lymphocyte function by spleen cell-derived and exogenous serotonin. *Immunopharmacol Immunotoxicol* **14,** 783.
9. Aune T.M., Golden H.W. & McGrath M. (1994) Inhibitors of serotonin synthesis and antagonists of serotonin 1A receptors inhibit T lymphocyte function *in vitro* and cell-mediated immunity *in vivo*. *J Immunol* **153,** 489.
10. Przegalinski E., Jaworska L. & Goembiowska K. (1992) The effect of p-chloroampetamine and p-chlorophenylalanine on the level of thyrotropin-releasing hormone and its receptors in some brain structures and lumbar spinal cord of the rat. *Neuropeptides* **23,** 19.
11. Young M.R., Newby M. & Wepsic H.T. (1987) Hematopoiesis and suppressor bone marrow cells in mice bearing large metastatic Lewis lung carcinoma tumors. *Cancer Res* **47,** 100.
12. Mitsuishi T., Akuzawa Y., Sato S. *et al.* (1993) Immunological properties of TtT/M-87 cell line established from murine pituitary tumor-associated macrophages. *Microbiol Immunol* **37,** 943.
13. Los G., de Weger R.A., van den Berg D.T.W.M., Sakkers R. & den Otter W. (1992) Macrophage infiltration in tumors and tumor-surrounding tissue: influence of serotonin and sensitized lymphocytes. *Cancer Immunol Immunother* **26,** 145.
14. Hellstrand K. & Hermodsson S. (1990) Enhancement of human natural killer cell cytotoxicity by serotonin: role of non-T/$CD16^+$ NK cells, accessory monocytes, and $5\text{-}HT_{1A}$ receptors. *Cell Immunol* **127,** 199.