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Quantitative Analysis of Contrasuppressor T Lymphocytes in Leprosy; Induction of Ia Antigens with Gamma Interferon[1]

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Leprosy is an infectious illness in which the immune response critically determines the clinical picture of the disease and its outcome ([6]). In tuberculoid leprosy (TT) there are few bacilli in the tissues. TT patients exhibit a strong cellular immune response against *Mycobacterium leprae* which causes the destruction of bacilli in the tissues. In lepromatous leprosy (LL), patients exhibit a selective immune anergy to antigens of *M. leprae*, and the bacilli cause a disseminated infection. LL patients have few lymphocytes in their lesions and do not respond to intradermal challenge with *M. leprae* antigens. When lymphocytes from TT patients are exposed *in vitro* to *M. leprae* antigens, they exhibit a high proliferative response; whereas in LL this does not occur ([1]). There is much evidence that this selective unresponsiveness seen in LL is due to an excess of suppressor T-cell function induced by *M. leprae* antigens ([14]). Mehra, *et al.* have delineated this suppressor-cell population as TH2+,CD8+, and have observed that depletion of this population in cell culture can restore the proliferative response of mononuclear cells to *M. leprae* antigens ([13]).

Contrasuppressor cells (Tcs) delineate a subset of T lymphocytes that reacts with CD8 antibodies and bind vicia villosa (VV) lectin ([11]). Ten to fifteen percent of Tcs are positive for Ia antigens ([11]). When T-helper cells are exposed to the action of Tcs, they become unresponsive to the signals of suppressor cells, and this results in an enhanced immune response to antigens ([16]). However, it is important to note that Tcs do not carry out helper functions. The number and functional status of Tcs have not been studied in many human diseases ([10]). It is necessary to assess this novel immunoregulatory function in conditions characterized by altered suppressor-cell function to further delineate the pathogenesis of these diseases. LL patients conspicuously exhibit aberrations in their immune suppressor function, but it is not clear whether this is also accompanied by abnormalities in the contrasuppressor circuit. In this work, we sought numerical abnormalities of Tcs lymphocytes in peripheral blood mononuclear cells (PBMC) of leprosy patients. In addition, we studied the expression of Ia (HLA-DR) and Tac (interleukin-2 receptor) antigens on these cells.

MATERIALS AND METHODS

Patients. Patients were classified according to the criteria of Ridley and Jopling ([18]). We studied 8 tuberculoid and 7 inactive lepromatous patients without evidence of leprosy reaction. All patients were receiving dapsone (DDS) but were not taking thalidomide for at least 2 months before the study. As controls, we studied 6 healthy contacts.

Isolation of PBMC and its subsets. Mononuclear cells were isolated from hep-

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arinized venous blood by Ficoll-Hypaque cushions as described previously ([2]). T-cell-enriched populations were obtained by nylon-wool columns as described elsewhere ([7]). CD8+ cells were obtained by treatment of T lymphocytes with OKT4 monoclonal antibody (Ortho Diagnostic Systems, Westwood, Massachusetts, U.S.A.) and a source of complement (normal rabbit serum) ([12]). CD8,VV+ enriched populations were isolated by panning on petri dishes coated with vicia villosa as described previously ([11]).

Effect of gamma interferon on the expression of Ia and Tac antigens by CD8,VV+ T lymphocytes. CD8,VV+ cells were incubated for 24 hr in the presence of 1000 U/ml of recombinant gamma interferon (rIFN-γ) (Biogen, Cambridge, Massachusetts, U.S.A.) at 37°C, 5% CO_2 and 100% humidity in RPMI 1640 medium supplemented with 5% fetal calf serum, 2 mM L-glutamine and antibiotics.

Immunofluorescence assay. T cells and their subsets were quantitated by immunofluorescence, as described previously ([5]). We used OKT3, OKT4, OKT8 (Ortho) and anti-Tac monoclonal antibodies. We also used a phycoerithrin-labeled anti-HLA-DR monoclonal antibody (Becton, Dickinson, Mountain View, California, U.S.A.). The VV+ cells were enumerated using fluoresceinated vicia villosa as described elsewhere ([8]). Double-labeling experiments were carried out to enumerate cells coexpressing Ia and Tac or Ia and VV-binding molecules. This was possible with the simultaneous use of phycoerithrin-labeled anti-Ia antibody and fluoresceinated VV lectin or using anti-Tac monoclonal antibody plus anti-mouse IgG labeled with fluorescein ([20]). We employed an epifluorescent microscope with the appropriate set of filters.

Statistical analysis. We compared the results obtained in different conditions within a group by the Wilcoxon rank test ([21]). To compare the results obtained by the different groups, we used the non-parametric Mann-Whitney *U* test ([21]).

RESULTS

As previously reported by our group and others ([5, 17]), we did not find significant differences in the percent of CD3, CD4, CD8 or Ia-positive cells in the PBMC of these inactive, nonreactional LL patients compared to TT or controls (Fig. 1). As expected, the same results were observed when we determined these cellular markers on T-cell-enriched preparations (Fig. 2); the percent of VV+ T lymphocytes was found to be similar in the three groups studied (Fig. 2).

When we determined the percent of Ia-positive cells in purified CD8,VV+ T lymphocytes, we found that LL patients exhibited a diminished percent compared to both TT or controls (Fig. 3). Double-labeling experiments performed with the CD8,VV+ population corroborated that VV+ cells account for the diminished expression of Ia (Fig. 3).

The effect of rIFN-γ on the expression of Ia and Tac antigens by CD8,VV+ cells is shown in Figures 4 and 5. Similar to what we observed in baseline conditions, Tcs cells from LL patients showed a diminished expression of Ia antigens after incubation with rIFN-γ. The expression of Tac antigen in baseline conditions by PBMC or Tcs cells was nil in all groups studied (data not shown). Approximately 10% to 15% of CD8+,VV-adherent lymphocytes expressed Tac antigen after incubation with rIF. As shown in Figure 4, the percent of Tac-positive cells was similar in the three groups studied.

When we compared the percent of Ia+ cells before and after treatment of the putative Tcs-cell population with rIFN-γ, we found a significant increase in the expression of this antigen in LL, TT and controls (Fig. 5). However, as stated above, this increase was greater in the controls and in tuberculoid patients compared to LL ($p < 0.05$).

DISCUSSION

The present work corroborates that inactive LL and TT patients do not possess numerical abnormalities in T-cell subpopulations of peripheral blood. As demonstrated by our results, this is also true for the percent of putative contrasuppressor cells, at least when these cells are detected using their capability to bind fluoresceinated vicia villosa lectin.

The low expression of Ia antigens in Tcs cells from LL patients is of interest. A poor

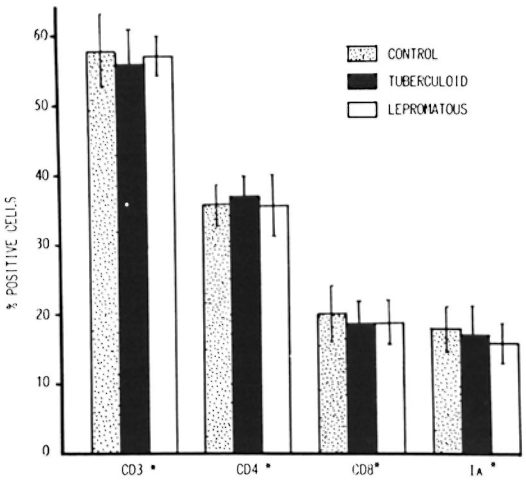

FIG. 1. T-cell subsets in peripheral blood mononuclear cells. *None of the groups are significantly different from the others. Lines within the bars express ±1 S.D.

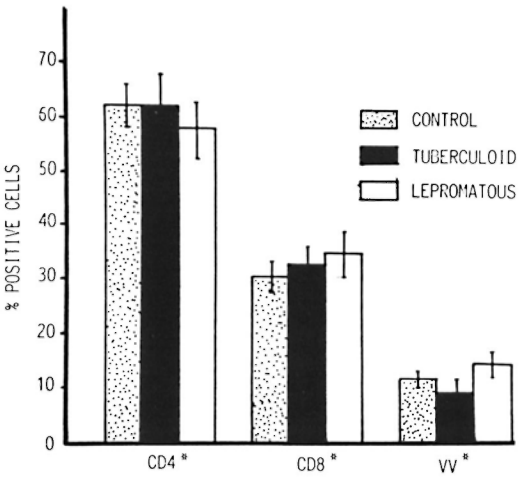

FIG. 2. Lymphocyte subsets in isolated T cells. *None of the groups are significantly different from the others. Lines within the bars express ±1 S.D.

expression of Ia antigens by CD8,VV+ cells might result in a defective interaction of Tcs cells with helper T cells. It has been suggested that an abnormal contrasuppressor-helper lymphocyte interaction might partially account for a defective immune response ([9]). Gershon, *et al.* have stated that the cellular interactions in the murine contrasuppressor circuit are restricted by class II MHC antigens ([4]). On the other hand, Lehner has found that human Tcs are also restricted by MHC antigens ([11]); consequently, a diminished percent of Ia+ Tcs cells might result in a defective interaction between Tcs and helper cells. Certainly, an abnormal Tcs function could be intimately related to the excess of suppressor function seen in LL patients. Thus, our findings suggest that LL patients might have abnormalities in the contrasuppressor cells that can be related to the immune anergy seen in lepromatous leprosy. However, it will be necessary to perform functional studies to further explore this point.

It has been well established that interferons, particularly immune (gamma) IFN, are capable of inducing the expression of MHC antigens on a variety of cell lines ([22]). Our results show that this lymphokine has the same effect on Tcs cells. Currently, it is not possible to establish how the interferon influences the *in vivo* expression of Ia antigens on Tcs cells. However, it is interesting that LL patients have a defective production of IFN-γ ([15]), and certainly this condition might account for the diminished baseline expression of Ia antigens by Tcs which we found.

Interestingly enough, IFN-γ can induce the expression of Tac antigen in Tcs cells. To our knowledge, there are no studies that assess the role of interleukin-2 (IL-2) on Tcs cells. However, it could be assumed that the IL-2 receptor in Tcs cells has the same role as in other T-cell subsets ([19]). The expression of IL-2 receptor in normal cells is transient, and the presence of antigen for its

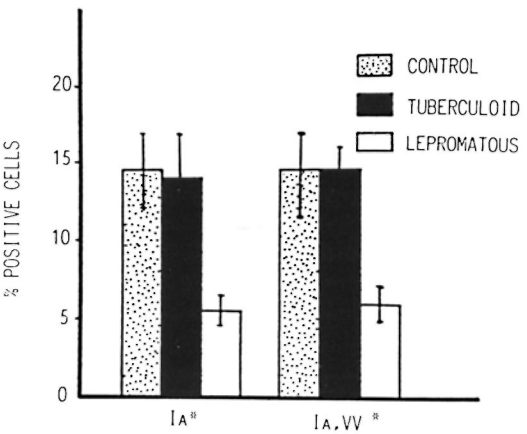

FIG. 3. Cell markers present on CD8+,vicia villosa-adherent T lymphocytes. * = $p < 0.05$ lepromatous compared to controls or tuberculoid. Lines within the bars express ±1 S.D.

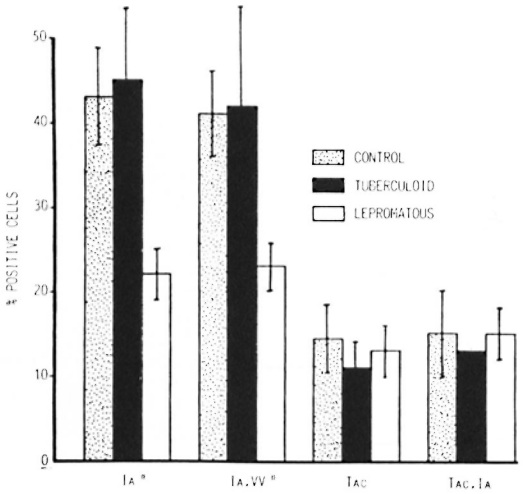

FIG. 4. Cell markers detected on CD8+,vicia villosa+ T lymphocytes following incubation with rIFN-γ. * = $p < 0.05$ lepromatous compared to control or tuberculoid. Lines within the bars represent ±1 S.D.

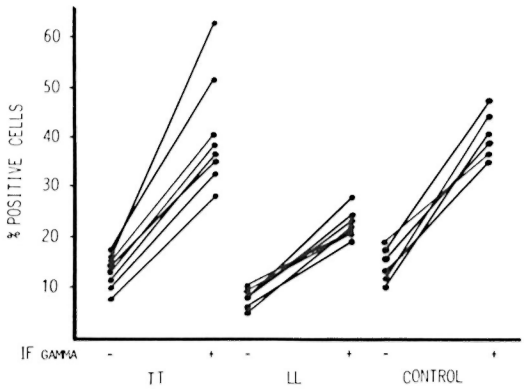

FIG. 5. Effect of rIFN-γ on the expression of Ia antigens on CD8+,vicia villosa+ T lymphocytes.

continuous expression is a requisite ([3]). It will be of interest to determine the kinetics of Tac expression in Tcs from LL patients and its modulation by *M. leprae* antigens. Our results indicate that Tcs cells from leprosy patients have the capability to express Tac antigens after rIFN-γ exposure. However, it is necessary to investigate whether these cells have a normal response to IL-2.

Our findings open the possibility to perform functional studies regarding the role of Tcs cells in the immune response against *M. leprae*. The depressed expression of Ia antigens on Tcs and the augmented suppressor function found in LL patients provide a unique model to further study the relevance of Tcs cells in the immune response.

SUMMARY

Lepromatous leprosy is characterized by immune anergy and abnormal suppressor T-cell function. Contrasuppressor cells are a subset of CD8+, vicia villosa-adherent T lymphocytes. T-contrasuppressor (Tcs) cells act on T-helper cells to cause them to become unresponsive to the action of T-suppressor cells. In 8 lepromatous (LL) and 7 tuberculoid (TT) patients, and 6 healthy contacts we studied the percent of the following lymphocyte subsets: CD3+, CD4+, CD8+, Ia+, vicia villosa+ (VV+), CD8, VV+, VV,Ia+, and Ia,Tac+. This was done in baseline status as well as post-stimulation with recombinant gamma interferon (rIFN-γ).

We found that peripheral blood mononuclear cells from LL and TT patients and controls exhibit a similar number of putative contrasuppressor lymphocytes (CD8,VV+ cells). However, in the contrasuppressor subset from LL patients we found a low percent of Ia+ ($p < 0.05$ compared to controls or TT). In the three groups studied, the rIFN-γ enhanced the percent of Ia+ lymphocytes in the CD8,VV+ cell subpopulation. However, the CD8,VV+ lymphocytes from LL patients, despite the effect of rIFN-γ, continue to have a low percent of Ia+ cells ($p < 0.05$ compared to controls or TT). These findings suggest that LL patients might have abnormalities in the contrasuppressor immune circuit. Future functional studies on the role of Tcs cells in the anergy seen in LL will be required in order to define the apparent dysfunction occurring in this disease.

RESUMEN

La lepra lepromatosa se caracteriza por anergia y por una función anormal de las células T supresoras (Ts). Las células contrasupresoras son una subpoblación de linfocitos T CD8+, adherentes a vicia villosa. Estas células contrasupresoras (Tcs) actúan sobre células T cooperadoras y las hacen no reactivas a la acción de las células Ts. En este trabajo se estudió la proporción de las subpoblaciones de linfocitos CD3+, CD4+, CD8+, Ia+, vicia villosa+ (VV+), CD8,VV+, VV,Ia+, y Tac Ia+, en 8 pacientes lepromatosos (LL), en 7 pacientes tuberculoides (TT) y en 6 controles sa-

nos. Esto se hizo tanto en el estado basal como después de la estimulación con interferón gamma recombinante (IFN-γ-r).

Se encontró que las células mononucleares de sangre periférica de los pacientes LL y TT y de los controles, exhibieron números similares de Lc Tcs (CD8,VV+). Sin embargo, en la subpoblación de células Tcs de los pacientes LL, se encontro un bajo porcentaje de célulals Ia+ ($p < 0.05$ comparado con los controles o con los pacientes TT). En los 3 grupos estudiados, el IFN-γ-r incrementó el porcentaje de linfocitos Ia+ en la subpoblación de células CD8,VV+, pero los linfocitos CD8,VV+ de los pacientes lepromatosos continuaron teniendo el porcentaje más bajo de células Ia+ ($p < 0.05$ comparado con los controles o con los pacientes TT). Estoa hallazgos sugieren que los pacientes LL podrían tener anormalidades en el circuito inmune contrasupresor. Para poder definir la aparente disfunción que ocurre en esta enfermedad, hacen falta más estudios sobre el papel de las células Tcs en la anergia mostrada por los pacientes LL.

RÉSUMÉ

La lèpre lépromateuse est caractérisée par une anergie immune et par une fonction anormale des cellules T du type suppresseur. Les cellules du type anti-suppresseur constituent un sous-groupe des cellules CD8+, les lymphocytes T qui adhèrent au vicia villosa. Les cellules T du type anti-suppresseur (Tcs) agissent sur les cellules T-adjuvantes pour les rendre incapables de répondre à l'action des cellules T du type suppresseur. Chez 8 malades lépromateux (LL) et chez 7 malades tuberculoides (TT), de même que chez 6 sujets témoins, on a étudié les proportions respectives des sous-groupes suivants de lymphocytes: CD3+, CD4+, CD8+, Ia+, vicia villosa+ (VV+), CD8,VV+, VV,Ia+, et Ia, Tac+. Cette étude a été menée à l'état de repos, et ensuite dans un état de post-stimulation par de l'interféron gamma recombinant (rIFN-γ).

On a observé que les cellules mononucléaires du sang périphérique, provenant de malades LL et TT, de même que de témoins, présentaient un nombre semblable de lymphocytes de type éventuellement anti-suppresseur (cellules CD8,VV+). Néanmoins, dans le sous-groupe de cellules de type anti-suppresseur provenant de malades LL, on a relevé un pourcentage plus faible d'Ia+ ($p < 0,05$ comparé aux valeurs notées chez les témoins ou les malades TT). Dans les trois groupes étudiés, l'interféron rIFN-γ a provoqué une proportion supérieure de lymphocytes Ia+ dans les sous-populations de cellules CD8,VV+. Néanmoins, les lymphocytes CD8,VV+ provenant de malades de LL ont toujours présenté une proportion faible de cellules Ia+ ($p < 0,05$, par comparaison avec les témoins et les malades TT, et ceci malgré l'effet de l'interféron). Cette observation suggère que les malades LL pourraient présenter des anomalies dans le circuit immunitaire anti-suppresseur. Des études complémentaires fonctionnelles sur le rôle des cellules Tcs dans l'anergie constatée chez les malades LL, sont nécessaires pour définir le dysfonctionnement apparent noté dans la lèpre.

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