

PN ABA-927

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE:

9/13/88

MEMORANDUM

TO: AID/PPC/CDIF/DI, room 209 SA-18
FROM: AID/SCI, Victoria Ose *VO*
SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. _____

5140

Final

Attachment

FINAL PROGRESS REPORT

"EVALUATION IN VITRO OF INTERLEUKIN 1 AND
INTERLEUKIN 2 AS POSSIBLE IMMUNOTHERAPEUTIC
AGENTS IN LEPROSY"

5.140

A RESEARCH PROJECT
USAID/PSTC PROGRAM
GRANT NO. 936-5542-G-00-5056-00

SUBMITTED BY
SANIT MAKONKAWKEYOON, Ph.D.
PROJECT LEADER
DEPARTMENT OF CLINICAL IMMUNOLOGY
FACULTY OF ASSOCIATED MEDICAL SCIENCES
CHIANG MAI UNIVERSITY
CHIANG MAI, THAILAND.

SEP 13 1988

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"EVALUATION IN VITRO OF INTERLEUKIN 1 AND
INTERLEUKIN 2 AS POSSIBLE IMMUNOTHERAPEUTIC
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PROJECT PROFILE

Country : Thailand.

Grant No. : 936-5542-G-00-5056-00.

Program : Program on Science and Technology Cooperation.

Project Title : Evaluation In Vitro of Interleukin 1 and
Interleukin 2 As Possible Immunotherapeutic
Agents in Leprosy.

Project Leader : Sanit Makonkawkeyoon, Ph.D.
Faculty of Associated Medical Sciences
Chiang Mai University
Chiang Mai, Thailand.

Co-investigators : Vicharn Vithayasai, M.D., Ph.D.
David M. Scollard, M.D., Ph.D.

Project Consultants : None

Authorized Officer : Sanit Makonkawkeyoon, Ph.D.

Total Project Budget : US \$ 150,000

Project Duration : June 1985-April 1988

Reporting Period : Final

1. BACKGROUND/INTRODUCTIONS:

Leprosy is a chronic infectious disease characterized by a unique spectrum of human immune responses to a single causative agent, *M. leprae*. Lepromatous patients, at one extreme, are almost totally but specifically non-responsive to *M. leprae* *in vivo* and *in vitro*; strong delayed hypersensitivity and cell-mediated immunity (CMI) mark tuberculoid patients at the other extreme, and most patients exhibit various intermediate (borderline) CMI responsiveness. Various defects in CMI have been demonstrated both *in vivo* and *in vitro*, especially in lepromatous patients, but their underlying cause and pathogenesis are unknown.

Recent developments in immunology and molecular biology now make it plausible to hypothesize that the range of aberrant CMI in leprosy is due to inadequate or inappropriate production of IL1 and/or IL2 by host mononuclear cells, resulting in a range of immunoregulatory disturbances as is observed in the immunopathologic spectrum of leprosy. If this is true, host immunity to *M. leprae* might be modified by immunotherapy to "normalize" IL1 and/or IL2 production or responsiveness; if false, it would exclude a number of presently known immunoregulatory mechanisms and re-direct work toward other immune cells and interactions. This hypothesis will be tested as follows:

1. Evaluation of IL1 and IL2 Production In Vitro :

Peripheral blood mononuclear leukocytes (PBML) will be obtained from healthy volunteers and various types of leprosy patients, classified clinically and pathologically. The PBML will be stimulated with concanavalin A, phytohemagglutinin, or *M. leprae* antigens to produce IL2, which will be assayed using an IL2-dependant cultured T cell line (CTLL-2). Adherent monocytes from patients' PBML will be stimulated with lipopolysaccharide (LPS) to produce IL1, which will be assayed using a mouse thymocyte bioassay.

2. Evaluation of Patient Lymphocyte Responsiveness to Exogenous IL1 and IL2 as Possible Immunomodulators In Vitro.

IL1 will be obtained from supernatants of LPS-stimulated cultures of normal human monocytes and from commercial sources. IL2 purified from products of a recombinant DNA bacterial strain is available from another laboratory. PBML of different types of leprosy patients, with or without these lymphokines, will be assayed for their ability to divide in response to mitogens, purified *M. leprae*-derived and/or synthesized antigens, and control antigens as a measure of T cell responsiveness. B-cell response to pokeweed mitogen will be measured using a modified reverse hemolytic plaque assay (PFC) and the secretion of IgG and IgM will be measured in culture supernatants using ELISA techniques.

3. Determination of Cell Types Required for IL2 Production.

If defects in IL2 production are demonstrated in the above studies, PBML from healthy persons and leprosy patients will be separated into monocyte, B-lymphocyte, T-helper and T-suppressor lymphocytes population by monoclonal antibody rosetting techniques. The different fractions will be assayed in different combinations to determine a) the cell types necessary for optimal IL2 production in response to mitogens and *M. leprae* antigens, and b) whether any observed defects in IL2 production by leprosy patients' lymphocytes are due to a consistent dysfunction of particular subset(s) or combinations. If such a defect is observed, relevant cell subsets from HLA-D-matched patients and healthy individuals will be co-cultured to see if such defects can be reversed.

2. OBJECTIVES:

The overall objectives of this research project are :

- (1) To determine the capability of monocytes and lymphocytes from leprosy patients and healthy subjects to produce IL1 and IL2 respectively.
- (2) To assess the effect, *in vitro*, of exogenous IL1 and IL2 on lymphocytes from leprosy patients.
- (3) Determination of cell types required for IL2 production.

3. MATERIALS AND METHODS:

PART I. PRODUCTION AND DETERMINATION OF IL1.

1. Separation and Purification of Human Monocytes.

Ten to twenty milliliters of heparinized blood is obtained from subjects. The blood is diluted 1:2 with sterile 0.01 M phosphate buffer saline (PBS) pH 7.2 and centrifuged on Ficoll-Hypaque. The peripheral blood mononuclear leukocytes (PBML) is collected, washed 3 times with RPMI 1640 media, cell concentrations adjusted with RPMI 1640 containing 100 units/ml penicillin, 100 ug/ml streptomycin, 2 mM L-glutamine and 10% heat inactivated fetal calf serum (FCS).

PBML 2×10^6 cells in 2.0 ml media are added onto a 35x10 mm. plastic plate, incubated at 37°C 5% CO₂ for 2-4 hrs. Non-adherent cells are washed off 3 times with 10% FCS-RPMI 1640. PBML, non-adherent and adherent cells are stained with nonspecific esterase stain (NSE). The exact number of monocytes adhering on the plate is calculated and the concentration of monocyte can be achieved by adding a certain amount of 10% FCS-RPMI 1640 media.

2. Production and Quantitation of IL1.

2.1 Production of IL1.

The adhered monocytes of normal or leprosy patients on plastic plates are adjusted to 2×10^5 cells/ml by adding various amounts of 10% FCS RPMI 1640 media. Twenty ug/ml of lipopolysaccharide (LPS) are added and incubated at 37°C, 5% CO₂ for 24 hours. Cell suspension is collected and centrifuged

and supernatants assayed for IL1.

2.2 Quantitation of IL1.

Thymocytes from 8 week old BALB/C inbred strain mice are used as the indicator cells in IL1 assay. Animals are killed by cervical dislocation and the thymus gland removed under sterile conditions. Thymocyte suspension is prepared by gently homogenizing the thymus gland in a sterile mesh with a sterile rubber plunger. The thymocytes are washed twice with IL1 media, counted, and then adjusted to 3×10^7 cells/ml.

Samples for IL1 assay are diluted 1:2, 1:4, 1:8 and 1:16 by IL1 media with a final volume of 600 ul for each dilution. One hundred microliters of thymocyte suspension are added to each tube, and thoroughly mixed. Thirty microliters of PHA-P (conc. 20 ug/ml) are added to each tube and mixed.

All IL1 sample dilutions are assayed in triplicate using 96 well tissue culture plates. Two hundred microliters of the above mentioned mixture are pipetted into each well. The microtiter tissue culture plates are incubated at 37 C 5% CO₂ for 72 hours. Thymidine incorporation into DNA is measured by adding 25 ul of tritiated thymidine (8 uCi/ml) into each well, mixed, and incubated 2 hrs.

The culture is harvested onto glass microfiber filters using a cell harvester. The glass microfiber filters are dried at room temperature overnight and transferred into scintillation counting vials containing 4.0 ml liquid scintillation fluid. All samples are counted in a liquid scintillation counter (LS 3801 Beckman

Liquid Scintillation Counter, Beckman Scientific Instruments, Inc., Fullerton, CA 92634 U.S.A.).

PART II. PRODUCTION AND DETERMINATION OF IL2.

1. Production of IL2.

PBML, 1×10^6 cells/ml are stimulated with 2 ug/ml PHA-P (Wellcome), or 40 ug/ml Con A (Sigma) or 10 ug/ml PPD (Connaught), incubated at 37 C 5% CO₂ 18 hours for PHA-P and Con A and 48 hours for PPD. Cell cultures are harvested and centrifuged, supernatant added with 10 mg/ml of α -methyl-D-mannoside, filtered sterile and stored at -70 C for IL2 assay.

2. Assay of IL2 Activity.

Tested samples are diluted in serial dilution 1:2, 1:4, 1:8, 1:16 to 1:256 with ILL media in 100 ul volume in each well of a 96 well tissue culture plates. One hundred microliters of twice washed CTLL-2 containing 8×10^3 cells/100 ul are added into each well, mixed, and incubated 24 hours. Fifty microliters of ³H-TdR containing 0.2 uCi/50 ul are added into each well and incubated for 18 hours. Cultures are harvested onto glass fiber filters and ³H-TdR incorporation is determined by a liquid scintillation counter (Beckman LS 3801).

PART III. PRODUCTION AND DETERMINATION OF INTERFERON.

1. Production of Interferon.

PBML, 1×10^6 cells/ml, stimulated with PHA-P 2 ug/ml or Con A 40 ug/ml or PPD 10 ug/ml at 37 C, 5% CO₂ for 18 hr for mitogens

and 48 hr for antigen. Supernatants are collected and kept at -70 C.

2. Assay of Interferon Activity.

Tested samples are diluted in serial two fold dilutions from 1:2, 1:4, to 1:1024 in 75 ul/well in a 96 well tissue culture plate. Well numbers 11 and 12 are virus and cell controls respectively. FL 5-1 cells are added, 75 ul/well (1.5×10^5 cells/ml). After 24 hours of incubation, 50 ul of Vesicular Stomatitis Virus (VSV) is inoculated into each well containing 2×10^3 plaque forming units (pfu), except the cell control wells which are filled with VSV free-culture media. After 24 hours of incubation, complete or 100% CPE is observed in virus control wells, while in cell control were FL 5-1 is completely intact.

Cells in each well is fixed with 10% formalin for 10 minutes and then stained with 0.4% crystal violet in 70% methanol for 5 minutes. Antiviral activity is expressed in interferon titer units. It is defined as the reciprocal of the highest dilution of the sample reduced by 50% of viral plaque. In our IFN assay, referent laboratory standard IFN were produced by PHA-P (2 ug/ml) stimulation of 2×10^6 cells/ml human PBML for 48 hours.

PART IV. DETERMINATION OF CELL TYPES REQUIRED FOR IL2 PRODUCTION

1. PBML. Heparinized blood was underlaid with Ficoll-Hypaque, centrifuged at 400xG for 30 min. Generally we get PBML with more than 95% purity.

2. Monocytes. Two milliliters of 1×10^6 /ml PBML were added onto a 15x60 mm plastic plate, incubated at 37 C 5% CO₂ for 2 hours. Non-adherent cells were washed out twice with warm RPMI medium. Adherent cells on the plastic plate were positive for nonspecific esterase staining more than 90%.

3. T cells. Ten milliliters of 2×10^6 /ml PBML were added into a Nylon Wool column, incubated at 37 C 5% CO₂ for 1 hour. Non-adherent cells were then eluted with warm RPMI medium. The resulting non-adherent cells were rosetted with AET-SRBC overnight at 4 C. The rosetted T cells were separated from non-rosetted cells by Ficoll-Hypaque gradient centrifugation. AET-SRBC were lysed by hypotonic ammonium chloride solution. When this AET-SRBC rosetting cells were mixed with AET-SRBC, more than 95% give rosette formation.

4. IL2 production. Two milliliters of 5×10^5 /ml T cells were added onto plastic plate containing autologous or heterologous monocytes. The cell mixtures were then stimulated with 2 ug of PHA-P for 18 hours. Supernatants from cell culture were assayed for IL2 activity by CTLL-2 proliferation.

PART V. DEVELOPMENT OF NEW TECHNIQUE.

At the present time we can only detect the level of IL2 in the culture fluid. However, if we have a technique which will be able to quantitate the numbers of cells producing IL2, we will open up new possibilities in the study of the immunoregulatory role of IL2 and IL2 production

We have developed a new technique which can quantitate the number of IL2-producing cells from rat spleen. We have also now finished adapting this technique for the human system. Our research article entitled, "Enumeration of Interleukin 2 - Producing Cells from Rat Spleen", has been published in "Asian Pacific Journal of Allergy and Immunology 5:129, 1987." The other research article entitled "Agar Plating Technique for Enumeration of IL2-Producing Cells in Human Peripheral Blood Mononuclear Leukocytes" has been submitted to the "Asian Pacific Journal of Allergy and Immunology"

4. RESULTS AND DISCUSSION

1. Separation and Purification of Monocytes.

The purity of monocytes isolated by adherent on plastic surface and evaluated by nonspecific esterase stain was quite satisfactory. In normal subjects, the purity of monocytes was $89.86 \pm 2.61\%$ and in leprosy patients $87.09 \pm 4.95\%$ (see detail in progress report number 3).

2. Production and Quantitation of IL1.

Optimal conditions for IL1 production of monocytes were determined. Optimal conditions for quantitative assay of IL1 using mouse thymocyte as an indicator system were also set up (see progress report number 2). Quantitative determination of IL1 from monocytes of normal subjects and leprosy patients were 622.4 ± 321.6 units/ml and 235.8 ± 226.9 units/ml respectively with

significantly different (see progress report number 3).

3. Production and Quantitation of IL2.

Optimal conditions for IL2 production of lymphocytes in PBML were determined. Appropriate conditions for quantitative assay of IL2 using CTLL-2 or mouse 3-day Con A blasts as indicator cells were also set up (see progress report numbers 1 and 2).

The level of IL2 from PBML of normal and leprosy patients were not significantly different when stimulated with mitogens, Con A or PHA-P (see progress report numbers 3 and 4) and figures 1 and 2. However, when PBML from normal (PPD + ve) and leprosy patients were stimulated with PPD, the level of IL2 was significantly high in normal than in patients (see progress report numbers 3 and 4) and figure 3.

When the new cases of leprosy patients were compared with normal subjects in the production of IL2 by PBML stimulated with Con A or PHA-P, there was no statistical different between the leprosy new cases and normal control (see figures 4, 5 and 7), however, when stimulated with PPD, there was significantly different in IL2 production (see figures 6 and 7). Comparison of long time treated cases with new cases of leprosy patients stimulated with Con A or PHA-P showed no statistical different (see figures 8 and 9). However, when treated cases were compared with new cases of leprosy patients in IL2 production stimulated with PPD, there was statistically different (see figure 10).

4. Production and Quantitation of Interferon.

Methods for stimulation and production of interferon from normal or leprosy patients were the same as in IL2 production. Bioassay of interferon was carried out by FL 5-1 cell line and VSV (Vesicular Stomatitis Virus). Interferon levels from BL/LL, BT/TT and normal controls was not significantly different when stimulated with PHA-P or Con A. However, when PBML was stimulated with PPD, the levels of interferon was significantly different between BL/LL vs normal and BT/TT vs normal (see progress report 2 number 4, and figures 11-13).

When level of interferon production from treated cases was compared with new cases of leprosy, there was no significant different between treated and new cases of BL/LL stimulated with Con A or PHA-P or PPD. Interferon level of new cases of lepromatous compared to normal controls, when Con A as stimulant $p > 0.05$, however, when PHA-P or PPD as stimulant resulting significant different ($p < 0.05$ and $p < 0.005$ respectively). After a long period of treatment, the treated lepromatous when compared to normal controls, the Con A and PHA-P stimulated interferon level was not significantly different, however, the PPD stimulated still showing different ($p > 0.05$, $p > 0.05$ and $p < 0.005$ respectively). (see figures 14-16).

5. Effects of IL1 on Lymphocyte Transformation of PBML.

Effects of IL1 on lymphocyte transformation was studied by incubating various concentrations of IL1 with PBML and with or without lepremin or PPD. IL1 seem to have no effect on

lymphocyte transformation of both normal and leprosy patients' PBML (see progress report number 4).

6. Effects of IL2 on Lymphocyte Transformation of PBML.

The effect of IL2 on lymphocyte transformation was studied by incubating various concentrations of IL2 with PBML in the presence or absence of lepromin or PPD. In normal subjects, IL2 itself was able to enhance lymphocyte transformation. However, IL2 had only little effect on lymphocyte transformation of the lepromin or PPD stimulated PBML (see figure 14 in progress report number 4). In leprosy patients, IL2 itself was able to enhance lymphocyte transformation in the high responder group, while this lymphokine was not be able to enhance lymphocyte transformation in the low responder group. IL2 also had a synergistic effect on lymphocyte transformation of lepromin or PPD stimulated PBML in the high responder group while little effect in the low responder group (see figures 15 and 17 in progress report number 4).

9 The combination of IL1 + IL2 on lymphocyte transformation of normal or leprosy patients' PBML was studied. IL1 + IL2 had enhancing effect on lymphocyte transformation of normal PBML and the high responder leprosy patient, while IL1 + IL2 had little effect on low responder's PBML (see figures 20 and 21 in progress report number 4).

7. Effects of IL1 or IL2 on IL2 Production of PBML.

Various concentrations of IL1 were incubated with PBML of normal or leprosy patients and levels of IL2 in the supernatant

fluid determined. IL1 did not have any enhancing effect on IL2 production of PBML from leprosy patients. However, when PBML from leprosy patients was incubated with IL2 then washed out IL2, the production of IL2 was enhanced by this treatment of IL2 (see results in progress report number 4).

8. Effect of IL2 on IL1 Production by Monocytes.

The effect of IL2 on IL1 production by monocytes was studied by incubating various concentrations of IL2 with monocytes. IL1 in supernatant fluid was determined by mouse thymocytes bioassay. Nine cases of lepromatous leprosy patients (LL) were evaluated in this study. IL2 showed enhancing activity to IL1 production by monocytes in all tested leprosy patients (see progress report number 4).

9. Cell Types Required for IL2 Production.

The requirement for IL2 production by normal T cells, monocytes, T cells mixed with autologous or heterologous monocytes were reported in progress report number 3. In this report, we showed that T lymphocytes was the major defect in most of leprosy patients in IL2 production while some leprosy patients had defect in monocyte function in helping IL2 production (see figures 17-23).

5. PUBLICATIONS OR MANUSCRIPTS RESULTING FROM THIS WORK

1. Makonkawkeyoon, S., Hirunpetcharat, C., Kasinrerak, W. and Vithayasai, V. (1987) Enumeration of interleukin 2-producing cells from rat spleen. *Asian Pacific Journal of Allergy and Immunology* 5: 129-136.
2. Hirunpetcharat, C., Kasinrerak, W. and Makonkawkeyoon, S. Agar plating technique for enumeration of IL2-producing cells in human peripheral blood mononuclear leukocytes. *Asian Pacific Journal of Allergy and Immunology* (submitted for publication).
3. Makonkawkeyoon, S. and Kasinrerak, W. *In vitro* suppression of interleukin 2 production by Mycobacterium leprae antigen. *Clin. Exp. Immunol.* (submitted for publication).
4. Makonkawkeyoon, S. and Kasinrerak, W. Separation of human suppressor and helper T cells by concanavalin A-coated sheep erythrocytes. *J. Clin. Immunol. Immunopathol.* (submitted for publication).
5. Kasinrerak, W., Hirunpetcharat, C. and Makonkawkeyoon, S. Optimal conditions for human interleukin 2 production. *Journal Medical Technologists Association of Thailand* (submitted for publication).
6. Chincharernpan, P., Hirunpetcharat, C. and Makonkawkeyoon, S. Quantitative assay for granulocyte activating factor (GAF). *Journal Medical Technologists Association of Thailand* (submitted for publication).

7. Makonkawkeyoon, S., Dettrairat, S. and Vithayasai, V. Evidences of immune aberrant of suppressor T lymphocytes in lepromatous leprosy (manuscript in preparation).
8. Makonkawkeyoon, S., Kasinrerk, W., Supajatura, V., Hirunpetcharat, C. and Vithayasai, V. Effects of exogenous IL1 and/or IL2 on IL1 and IL2 production in vitro in leprosy (manuscript in preparation).
9. Kasinrerk, W., Supajatura, V., Hirunpetcharat, C., Makonkawkeyoon S. and Vithayasai, V. Interleukin 1, interleukin 2 and interferon production in leprosy patients (manuscript in preparation).
10. Makonkawkeyoon, S., Kasinrerk, W. and Rungruengthanakit, K. Regulation of interleukin 2 production in leprosy patients (manuscript in preparation).

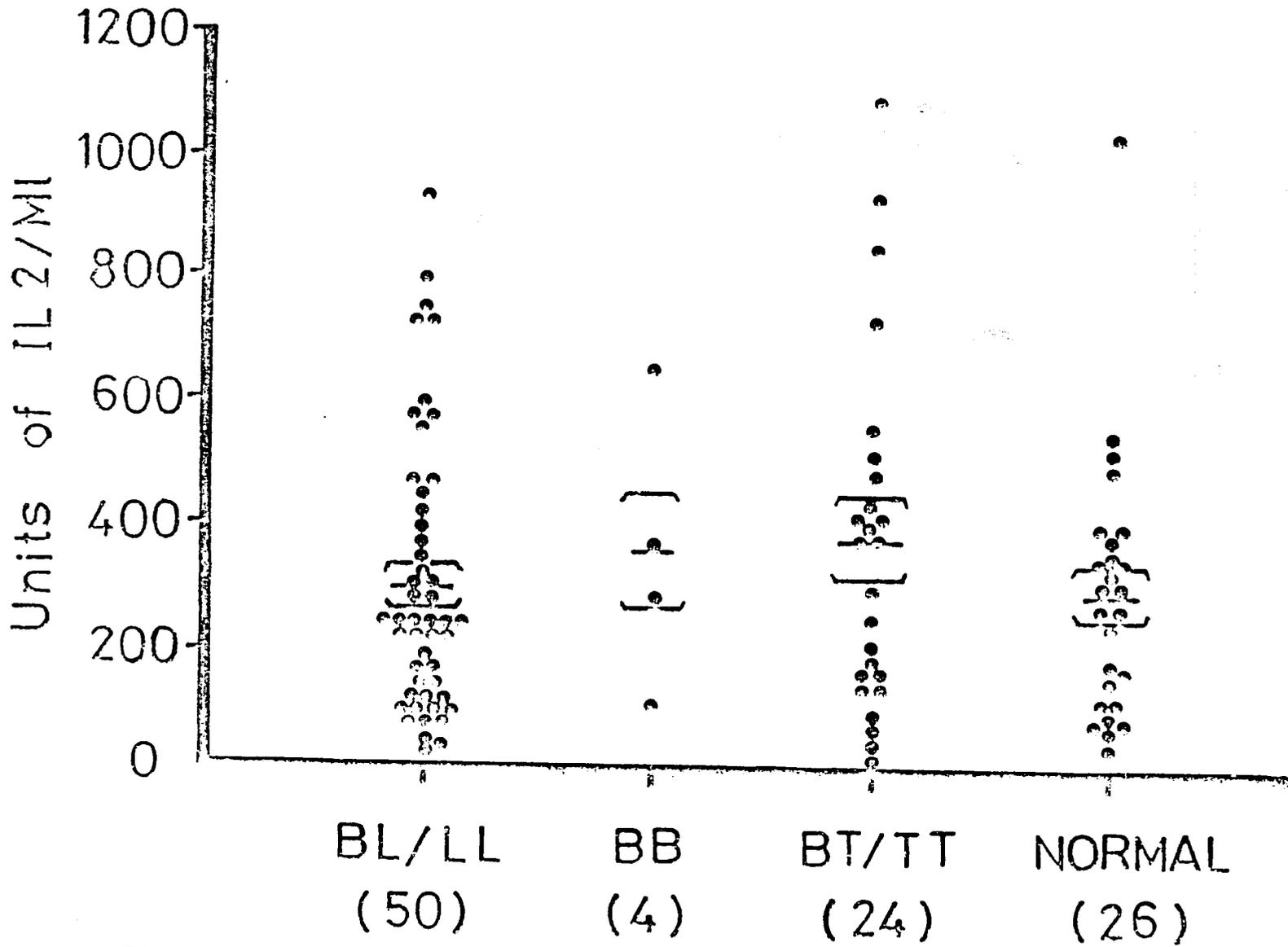


Figure 1. Interleukin 2 production by PBML of leprosy and normal subjects stimulated with 40 ug/ml Con A ($\bar{x} \pm S.E.$).

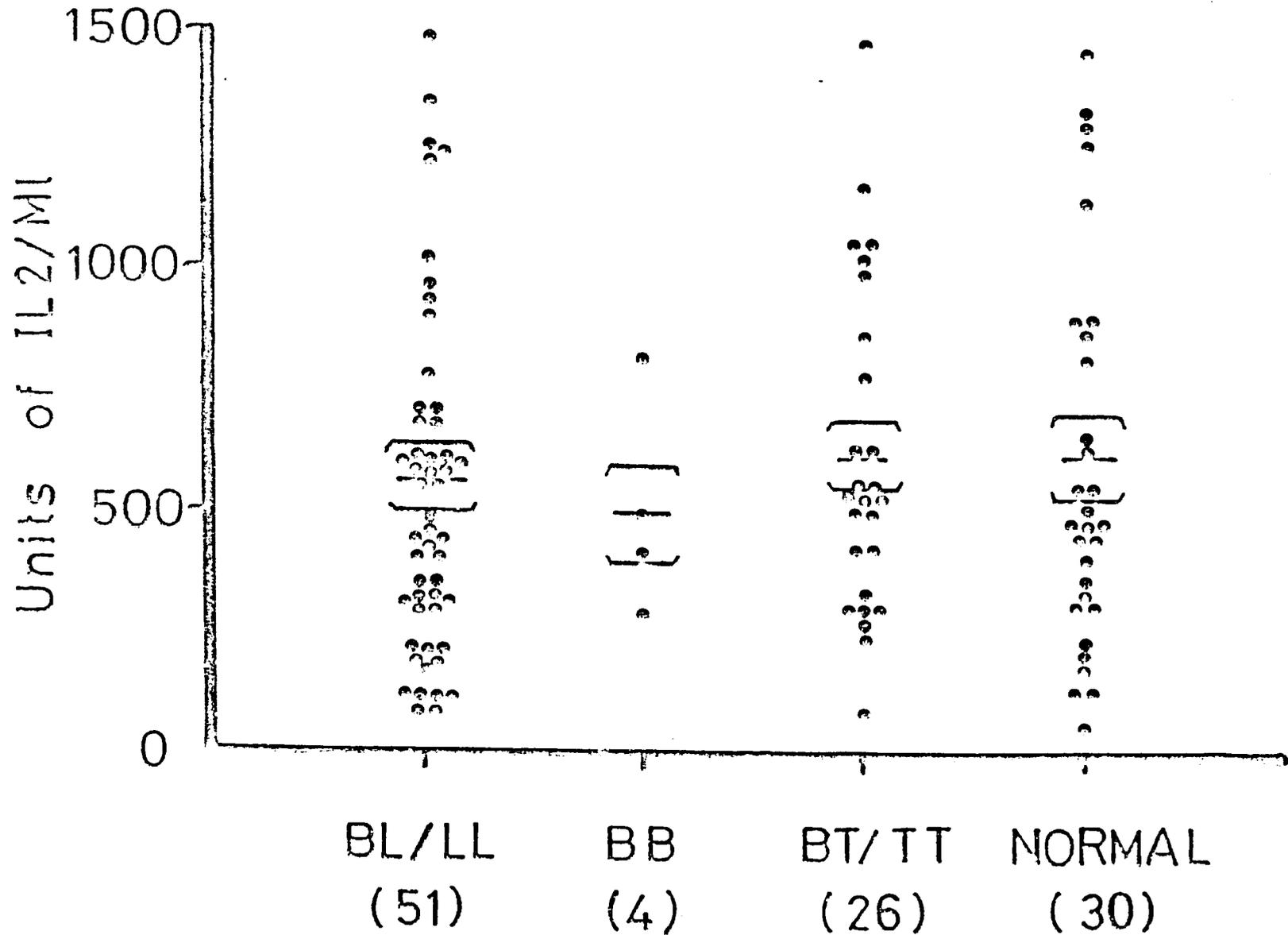


Figure 2. Interleukin 2 production by PBML of leprosy and normal subjects stimulated with 2 ug/ml PHA-P (\equiv = Mean \pm S.E.).

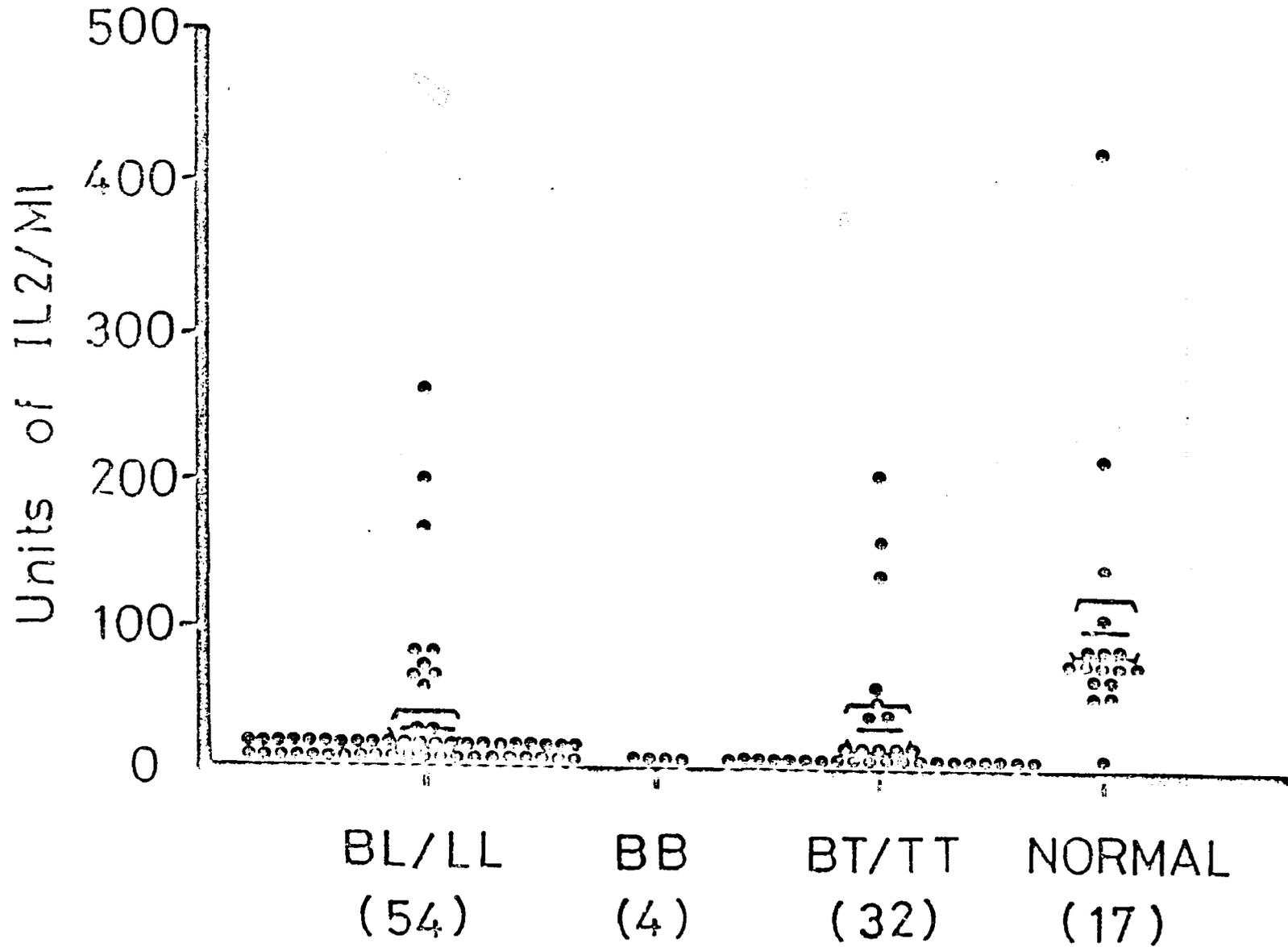


Figure 3. Interleukin 2 production by PBML of leprosy and normal subjects stimulated with 10 ug/ml PPD (\equiv = Mean \pm S.E.).

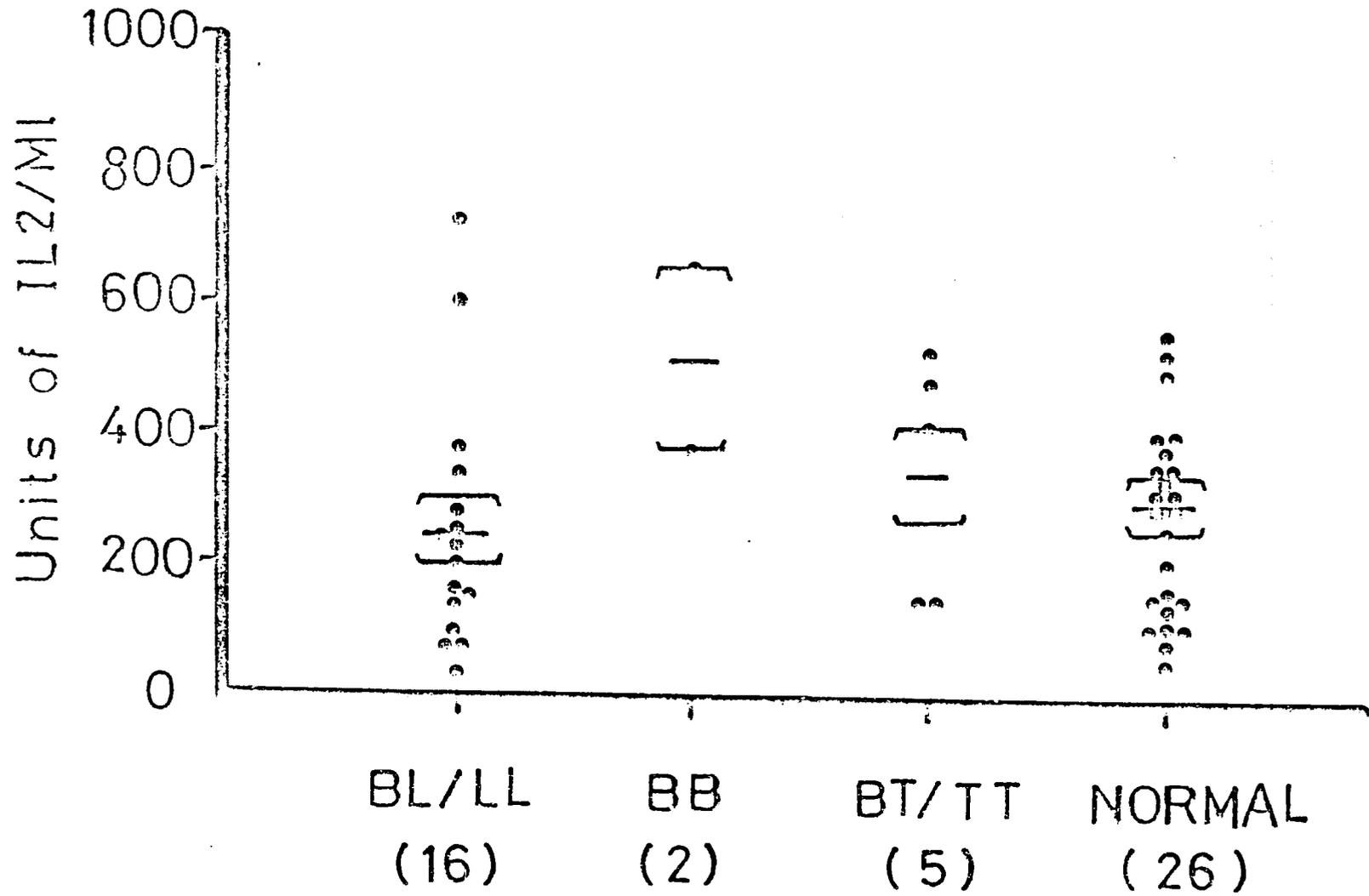


Figure 4. Interleukin 2 production by PBML of new cases of leprosy compared to normal subjects stimulated with Con A (\bar{x} = Mean \pm S.E.).

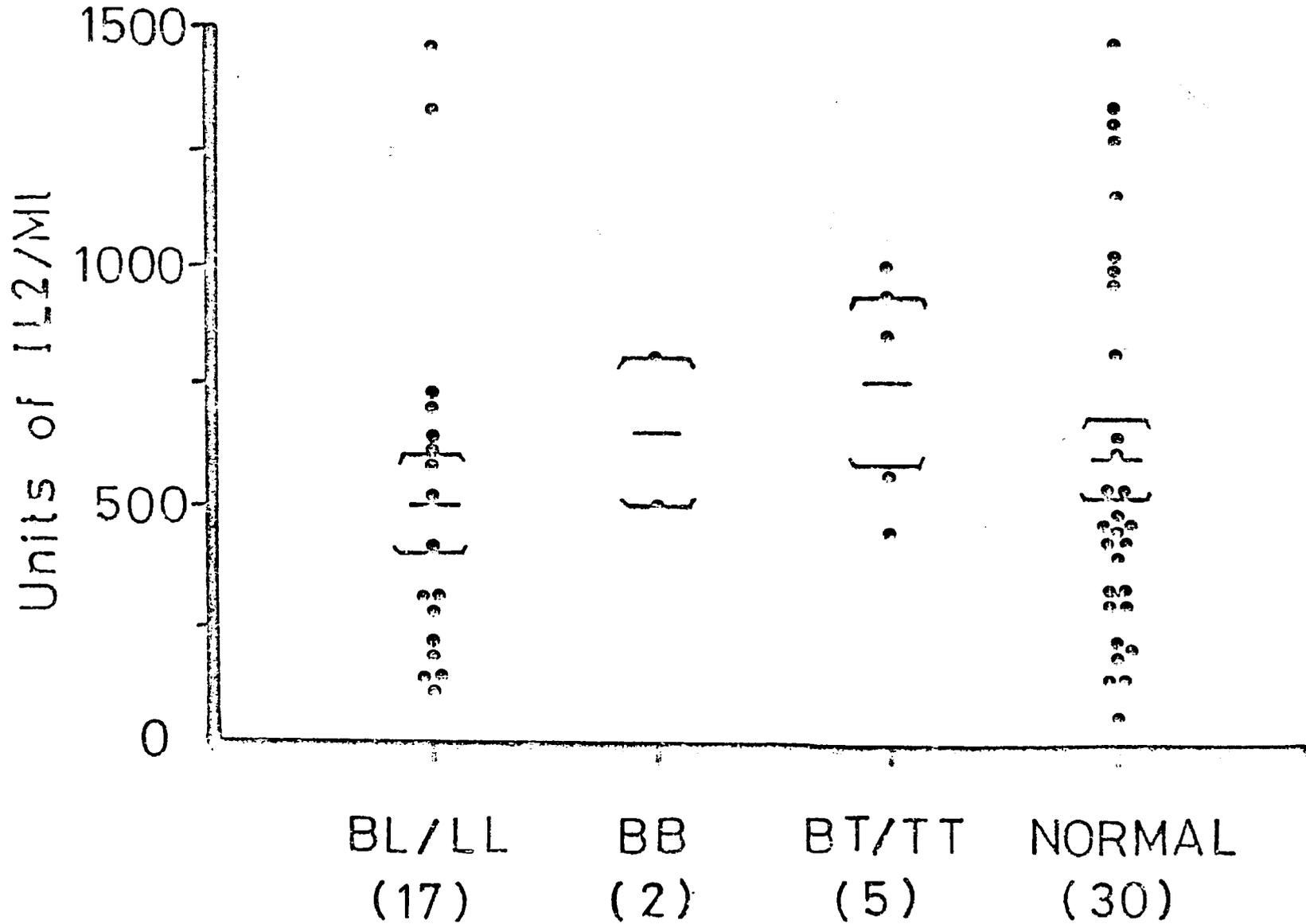


Figure 5. Interleukin 2 production by PBML of new cases of leprosy compared to normal subjects stimulated with PHA-P ($\bar{x} \pm S.E.$).

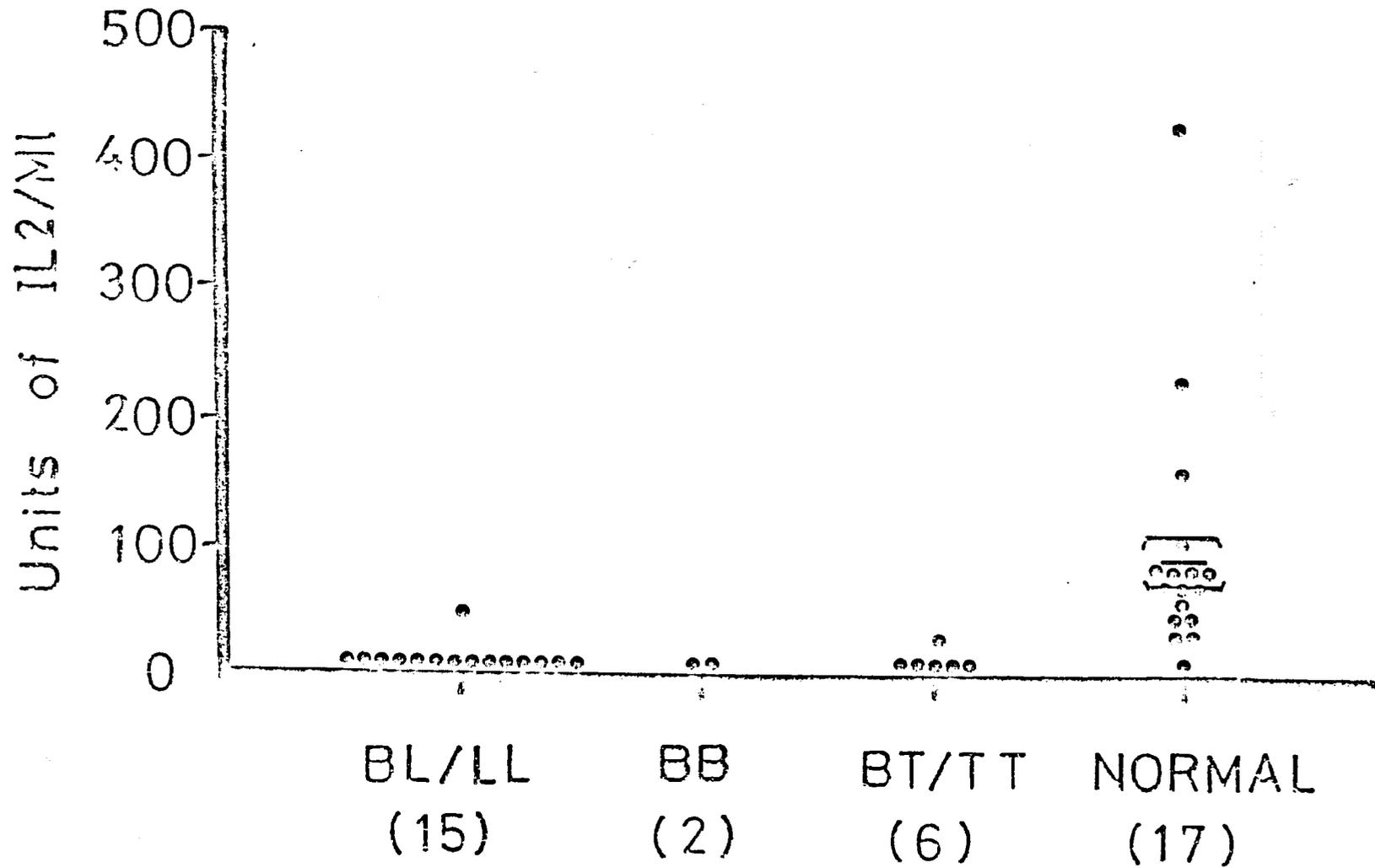


Figure 6. Interleukin 2 production by PBML of new cases of leprosy compared to normal subjects stimulated with PPD (\bar{x} = Mean \pm S.E.).

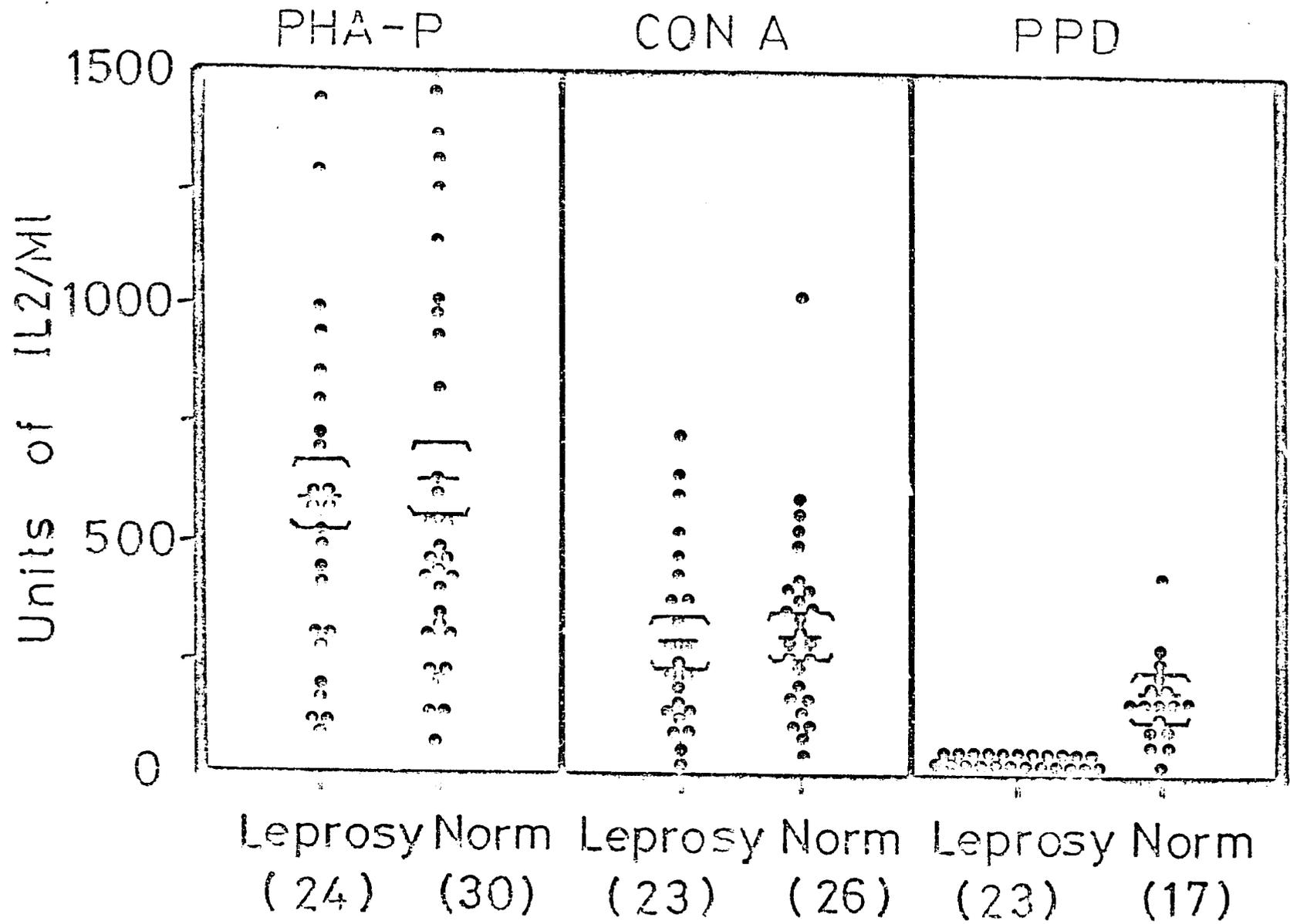


Figure 7. Interleukin 2 production by PBML of new cases of leprosy compared to normal subjects stimulated with PHA-P, Con A and PPD (\bar{x} = Mean \pm S.E.).

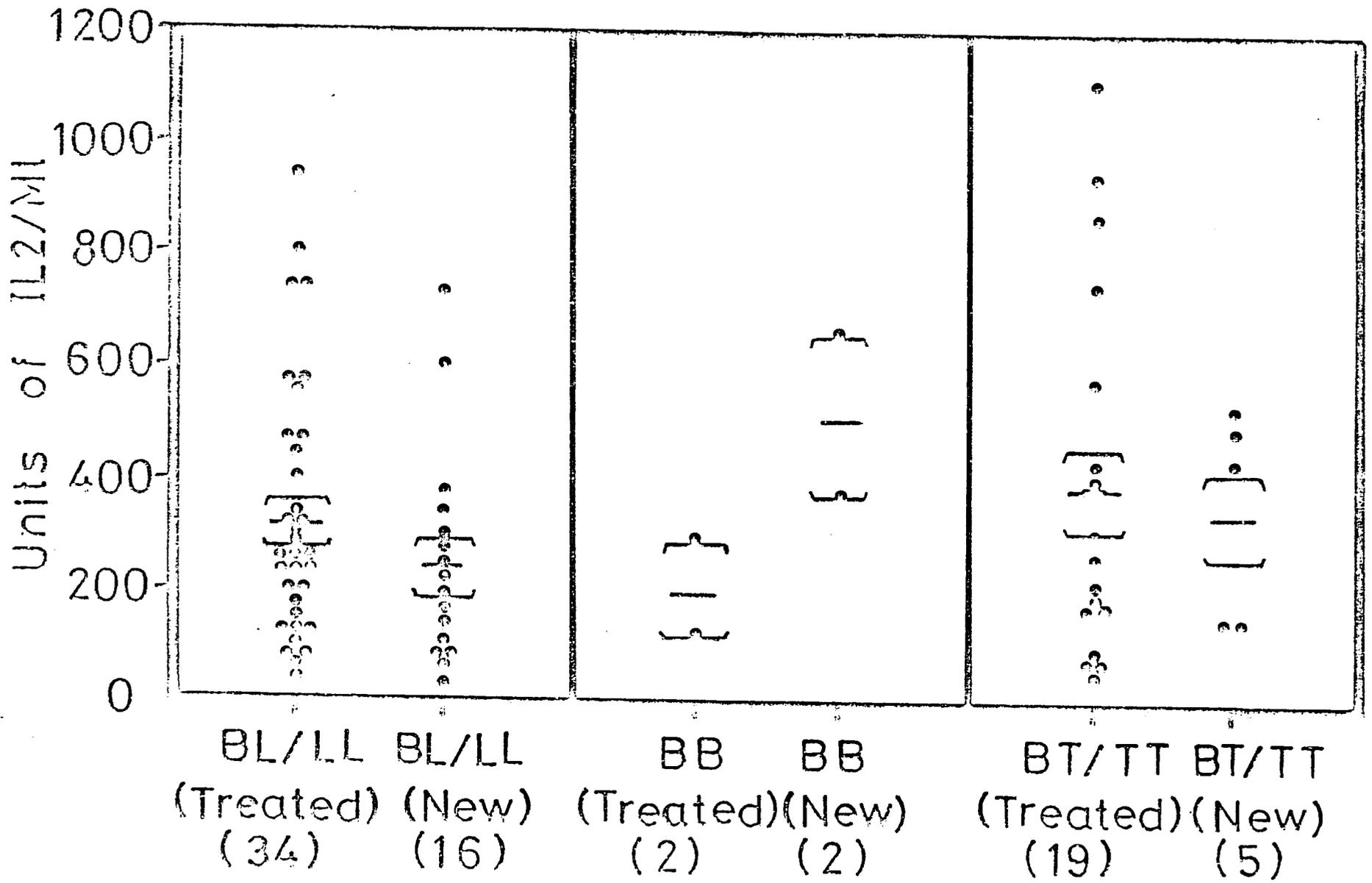


Figure 8. Interleukin 2 production by PEML of new cases compared to treated cases of leprosy stimulated with Con A ($\bar{x} \pm S.E.$; $p > 0.05$ in all types of leprosy).

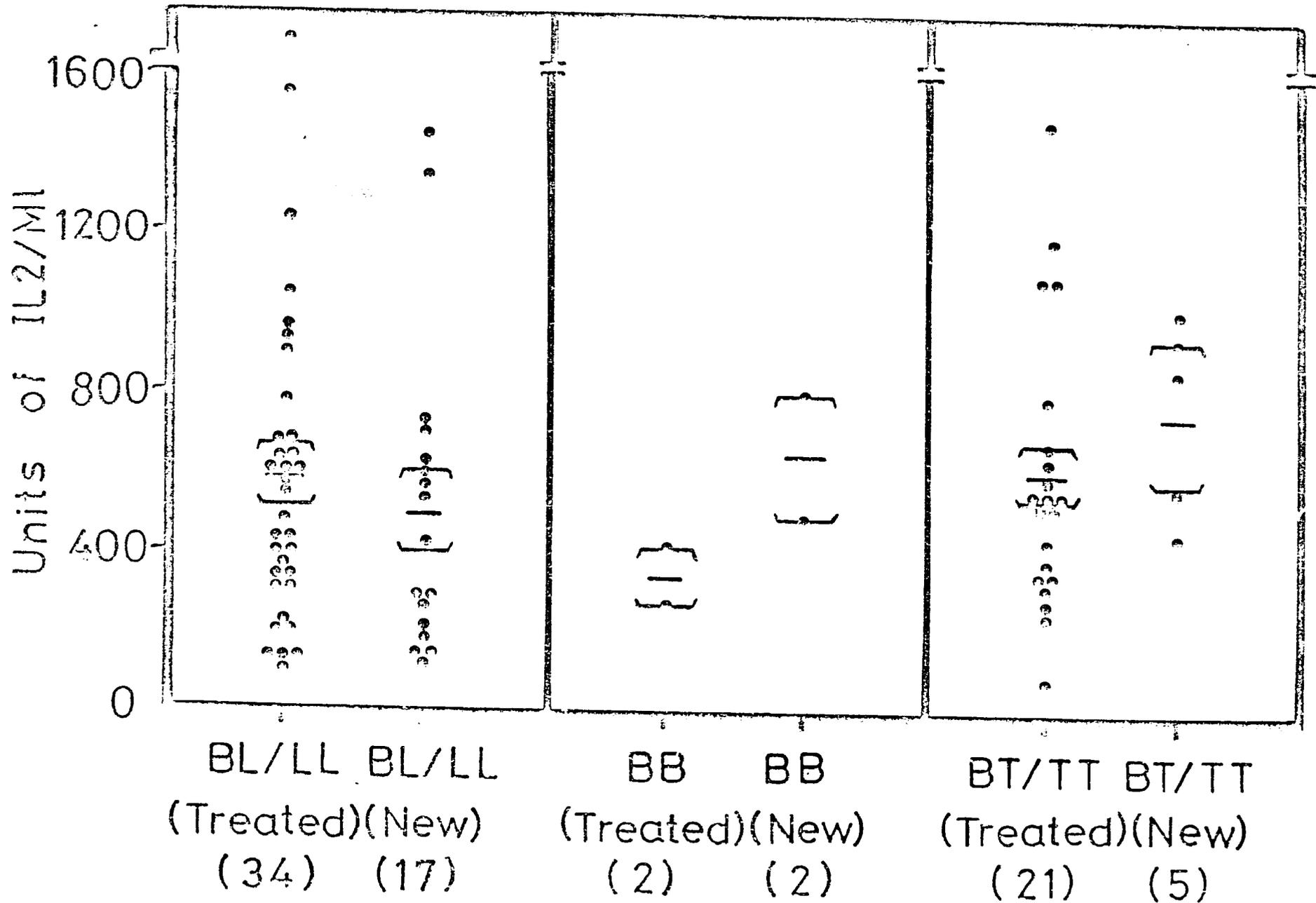


Figure 9. Interleukin 2 production by PBML of new cases compared to treated cases of leprosy stimulated with PH -P ($\bar{x} \pm s.e.$ = Mean \pm S.E.; $p > 0.05$ in all types of leprosy).

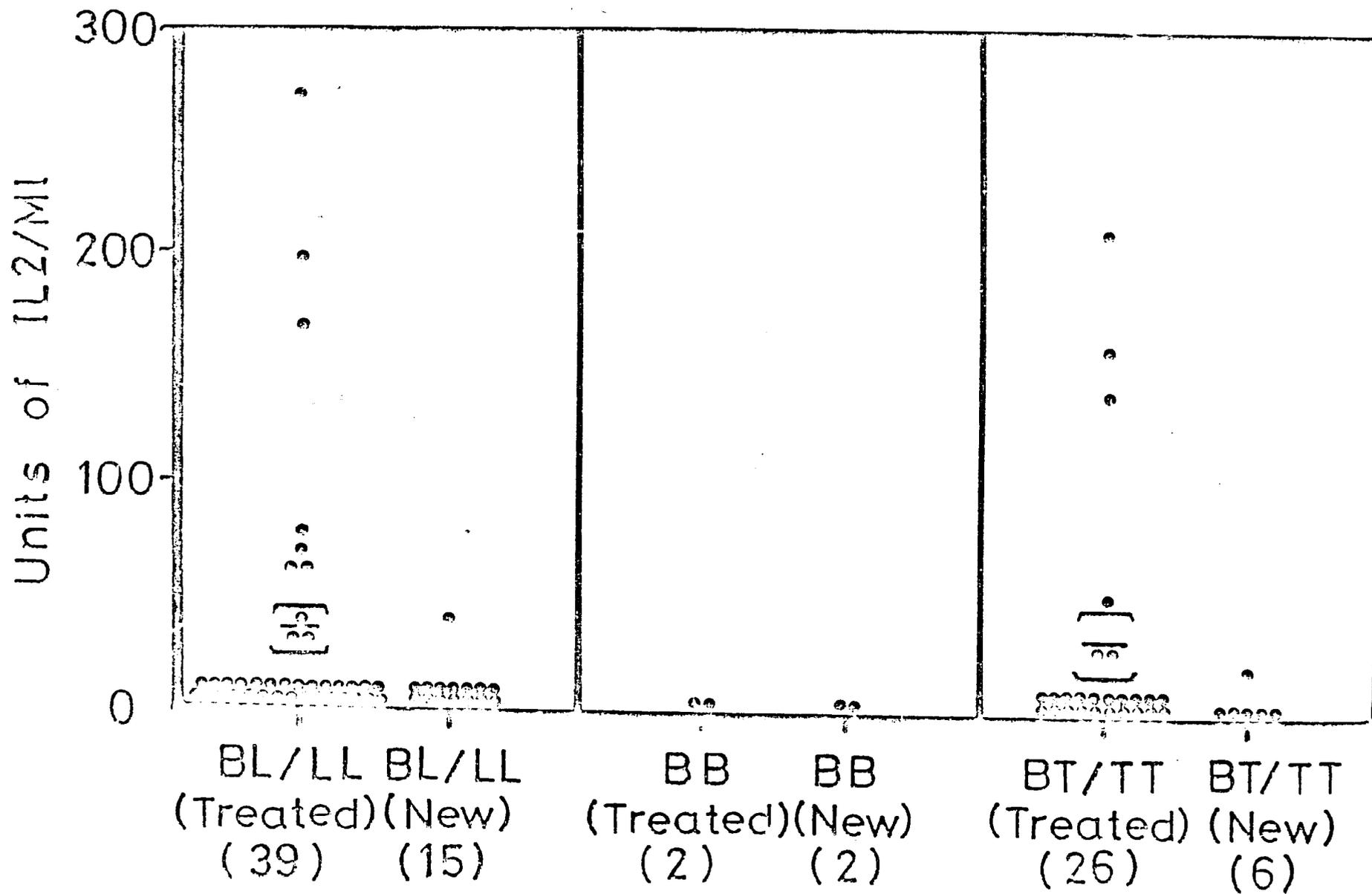


Figure 10. Interleukin 2 production by PBML of new cases compared to treated cases of leprosy stimulated with PPD ($\bar{x} \pm s.e.$ = Mean \pm S.E.; $p < 0.0125$ in BL/LL and $p < 0.05$ in BT/TT).

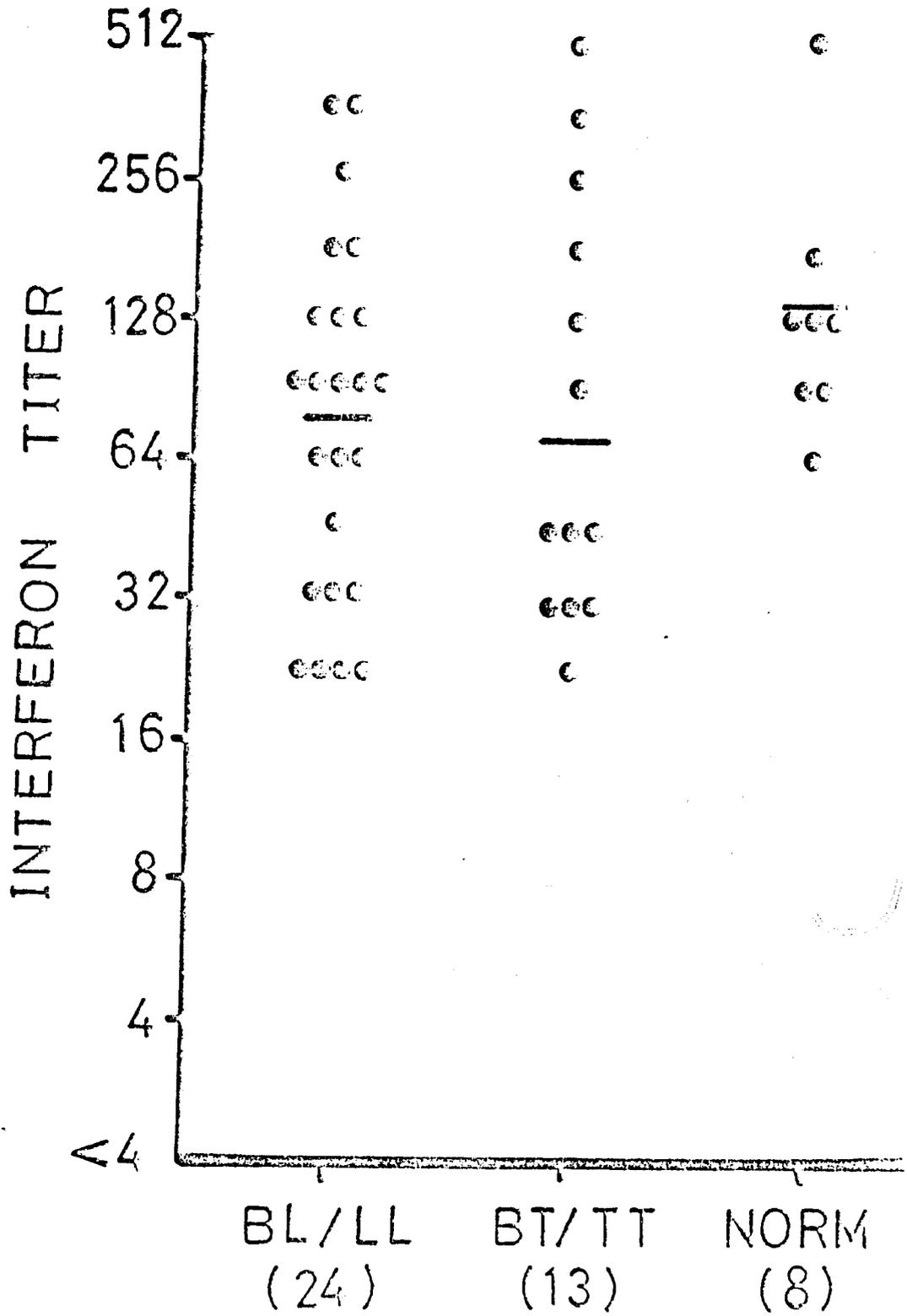


Figure 11. Interferon production by PBML of leprosy and normal subjects stimulated with 2 ug/ml PHA-P (— = Mean; $p > 0.05$ in BL/LL vs normal and BT/TT vs normal).

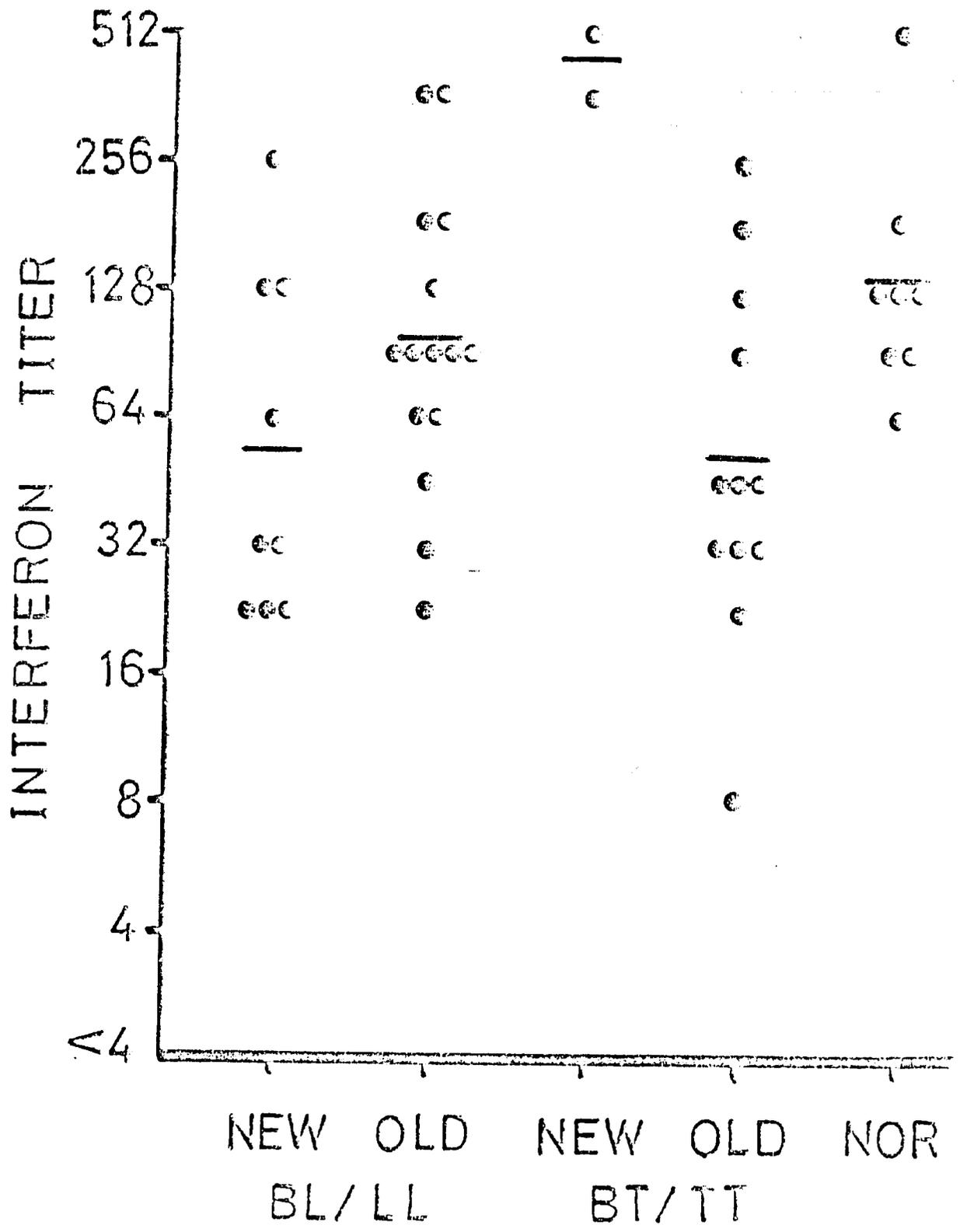


Figure 14. Interferon production by PBML of new and treated cases of leprosy compared to normal controls stimulated with PHA-P (— = Mean; $p > 0.05$ in treated BL/LL or BT/TT vs normal; $p < 0.05$ in new cases of BL/LL or BT/TT vs normal).

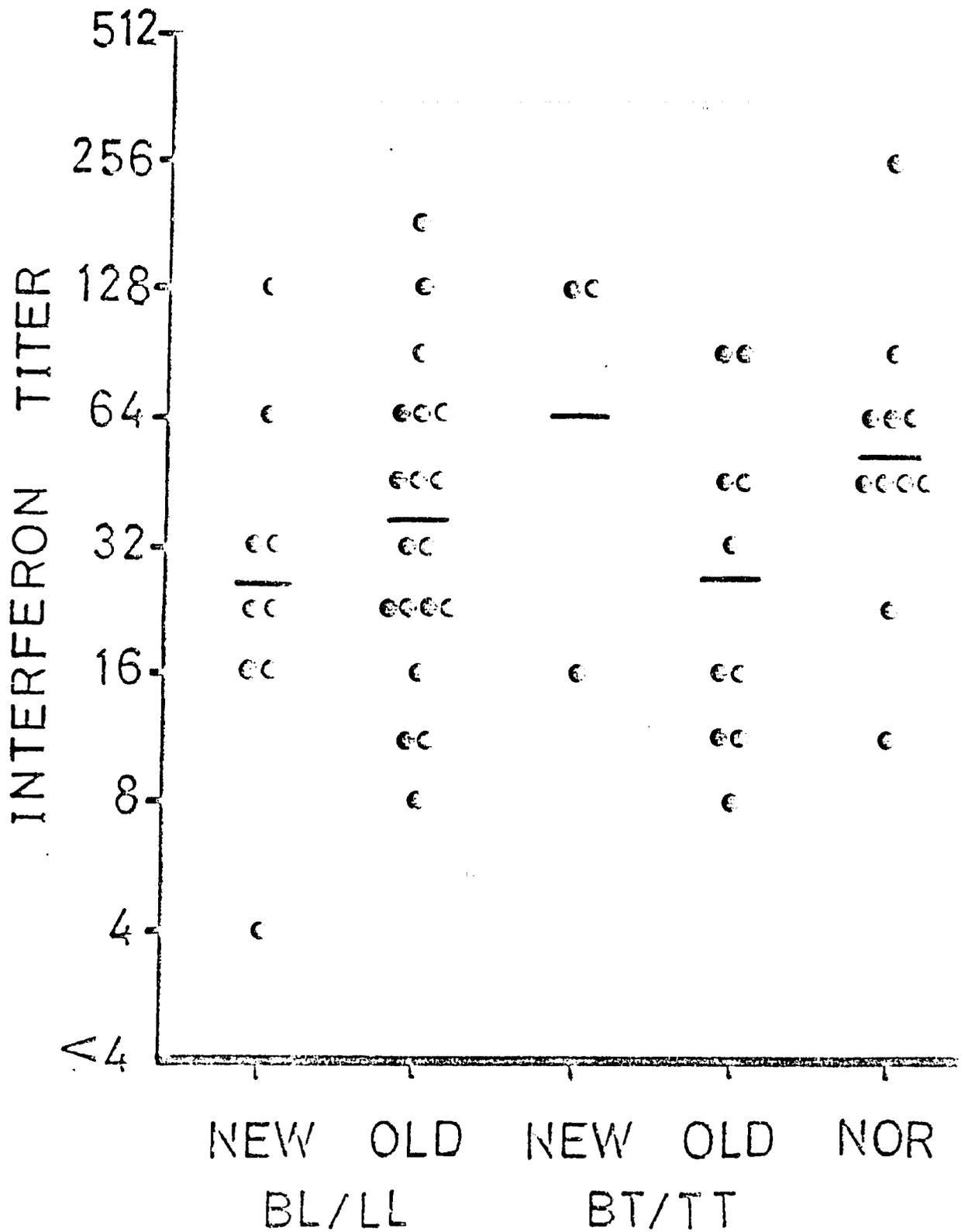


Figure 15. Interferon production by PBML of new and treated cases of leprosy compared to normal controls stimulated with Con A (— = Mean; $p > 0.05$ in treated BL/LL or BT/TT vs normal; $p < 0.005$ in new cases of BL/LL vs normal).

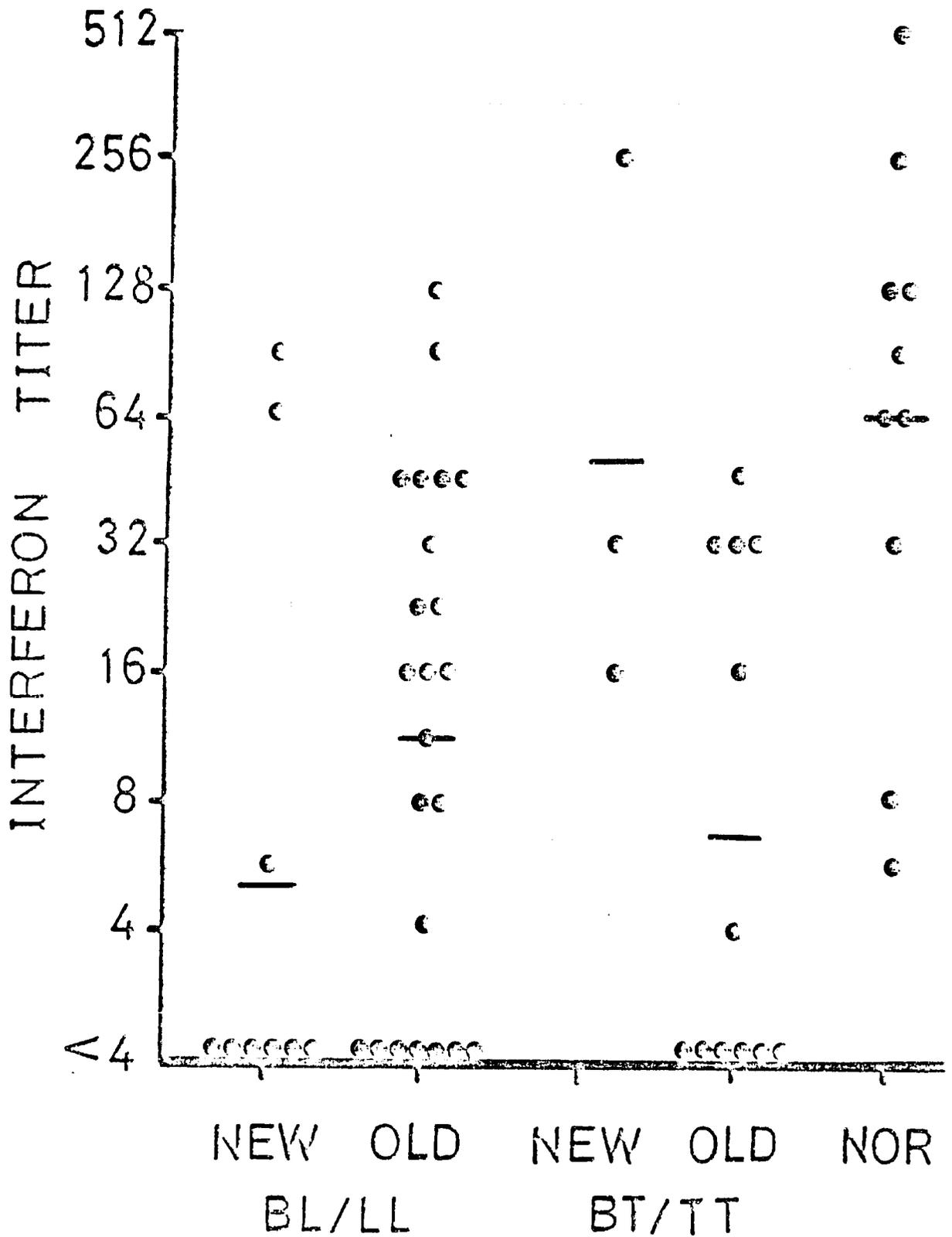


Figure 16. Interferon production by PBML of new and treated cases of leprosy compared to normal controls stimulated with PPD (— = Mean; $p < 0.005$ in treated BL/LL or BT/TT vs normal; $p < 0.005$ in new cases of BL/LL vs normal).

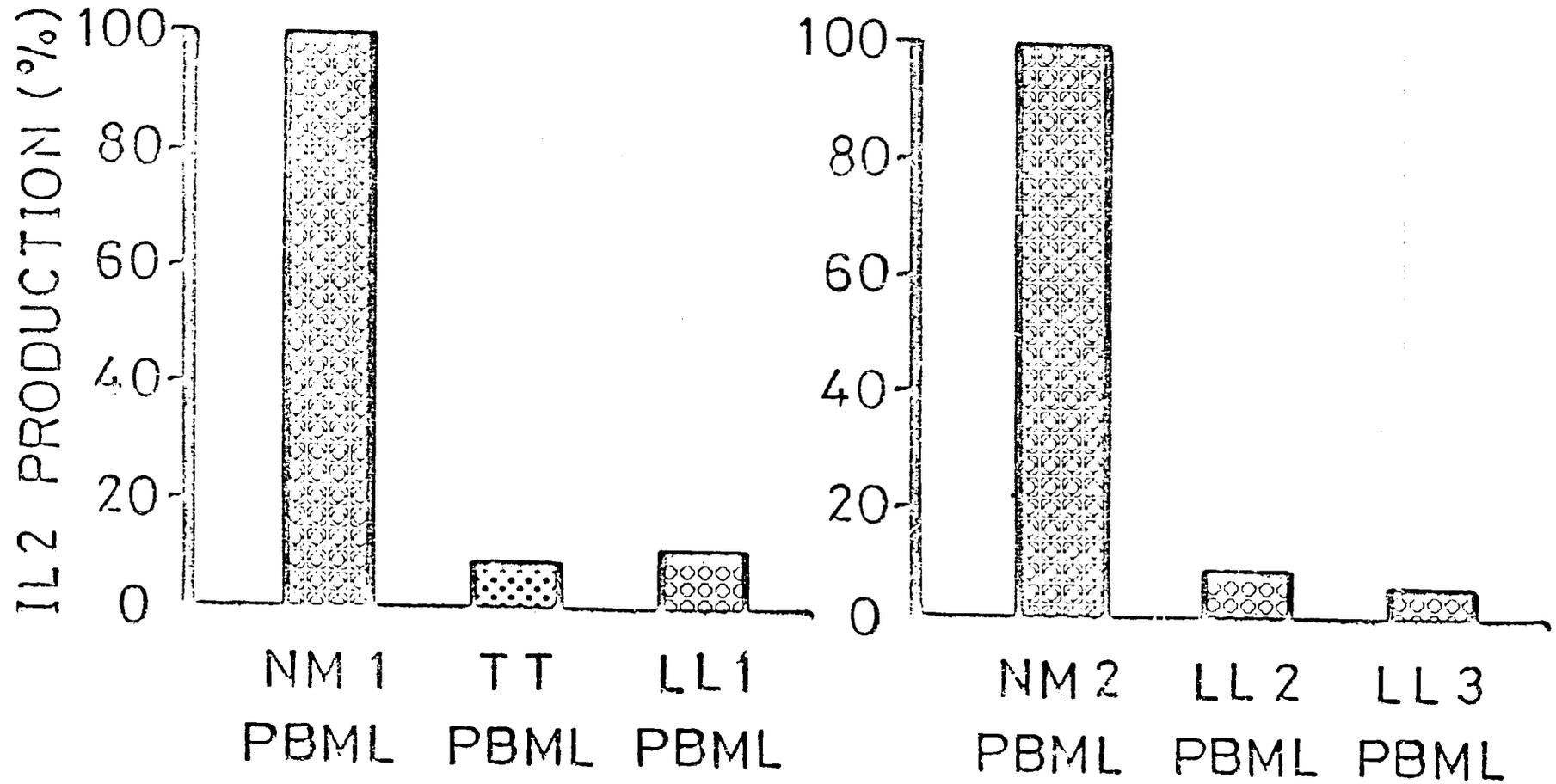


Figure 17. Production of interleukin 2 by PBML of normal subjects (NM1 and NM2), tuberculoid (TT) and lepromatous leprosy (LL1, LL2 and LL3).

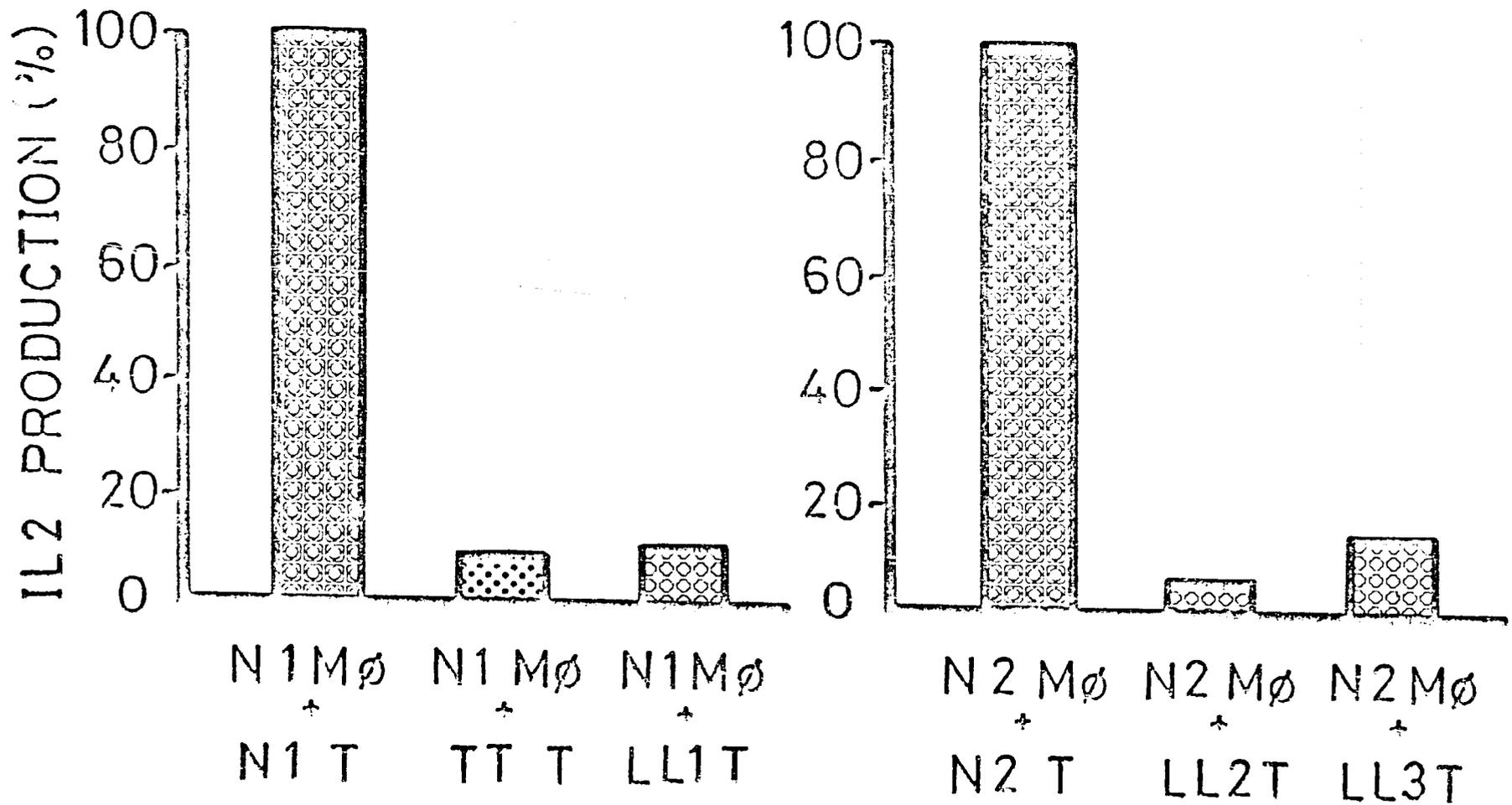


Figure 18. Production of interleukin 2 by mixed cultures of monocytes with homologous or heterologous T lymphocytes (N = normal; Mø = monocytes; TT T = T lymphocytes from TT; LL T = T lymphocytes from LL).

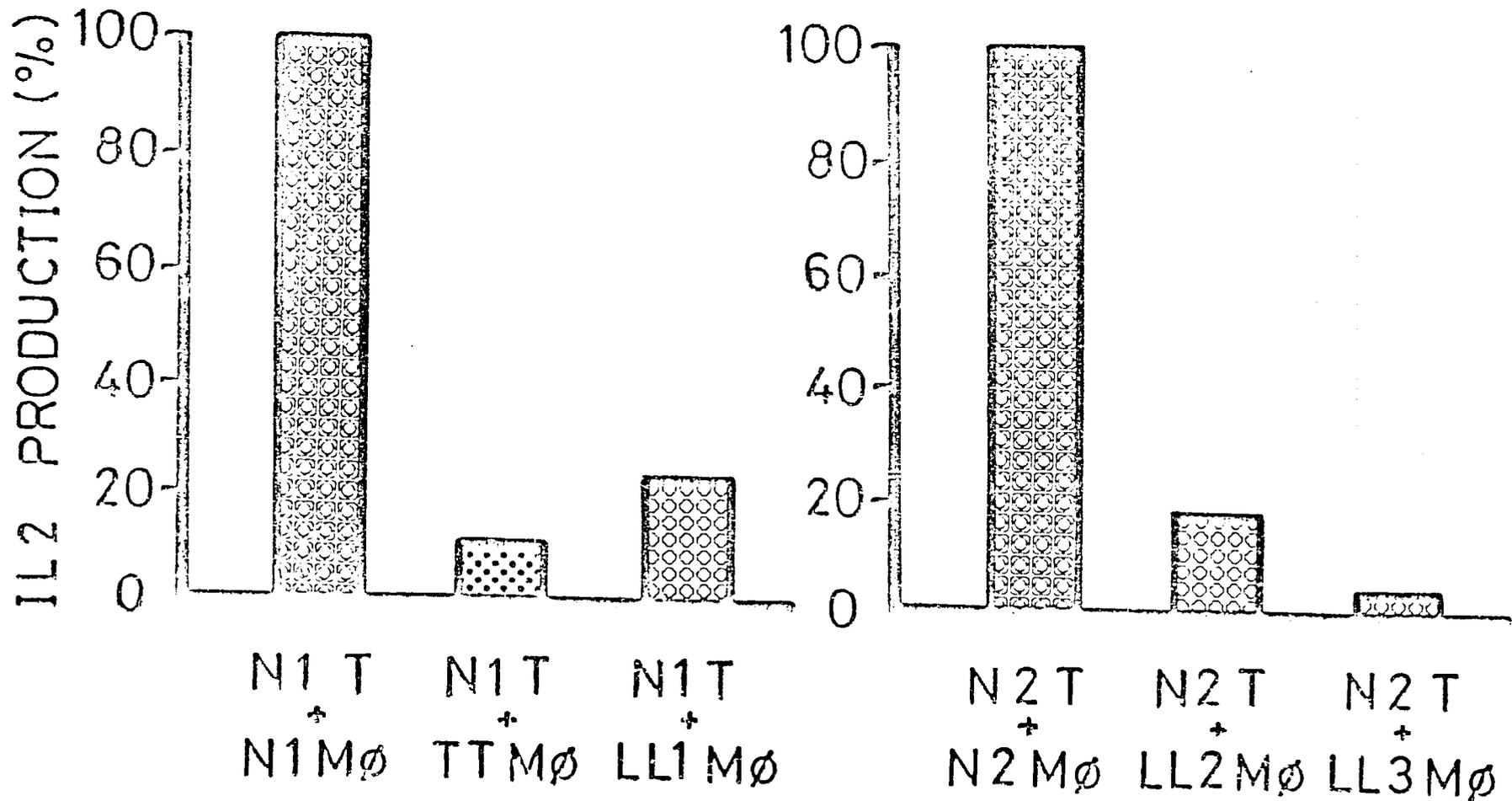


Figure 19. Production of interleukin 2 by mixed cultures of T lymphocytes with homologous or heterologous monocytes (N = normal; T = T lymphocytes; TT Mø = monocytes from TT; LL Mø = monocytes from LL).

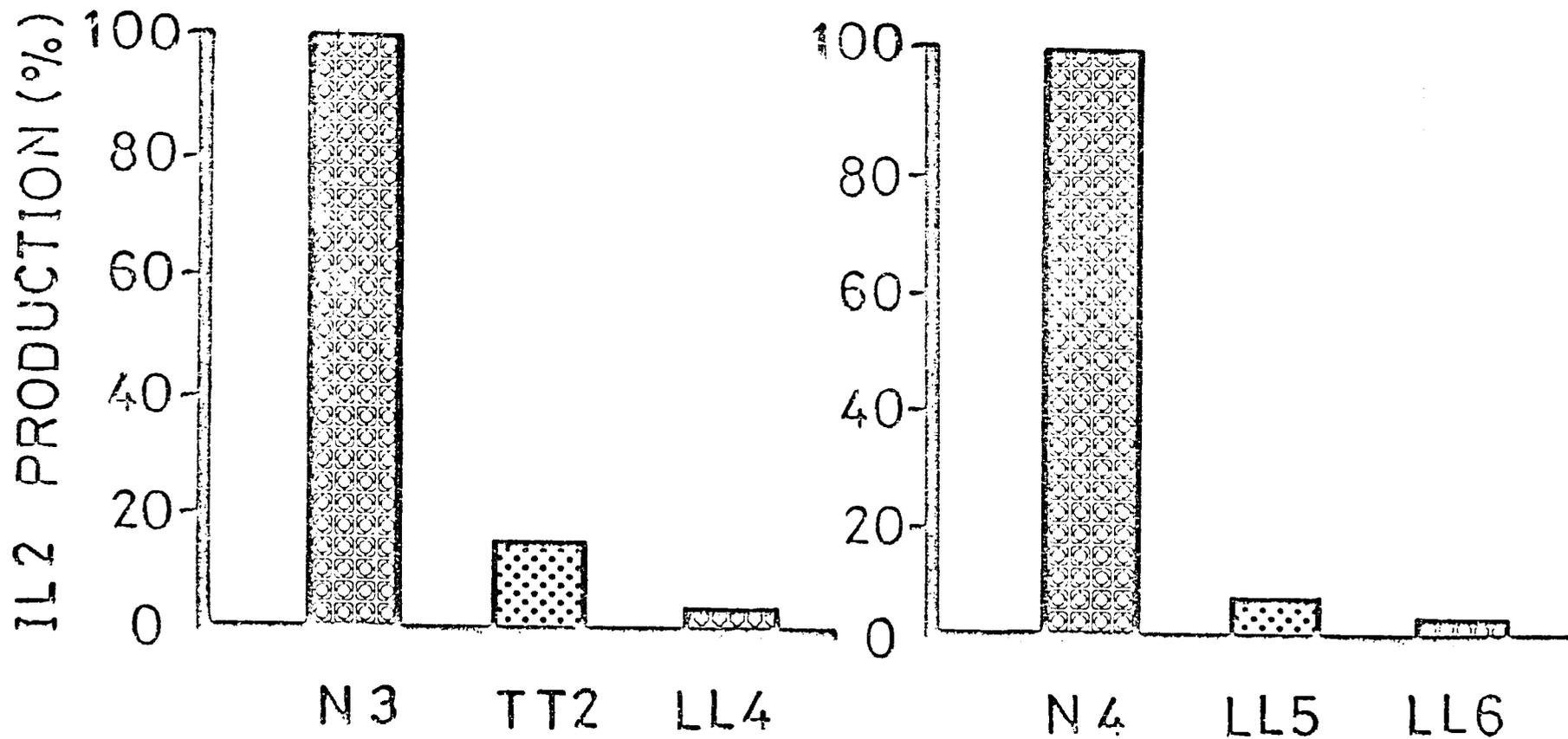


Figure 20. Production of interleukin 2 by PBML of normal subjects (N3 and N4), tuberculoid (TT2) and lepromatous leprosy (LL4, LL5 and LL6).

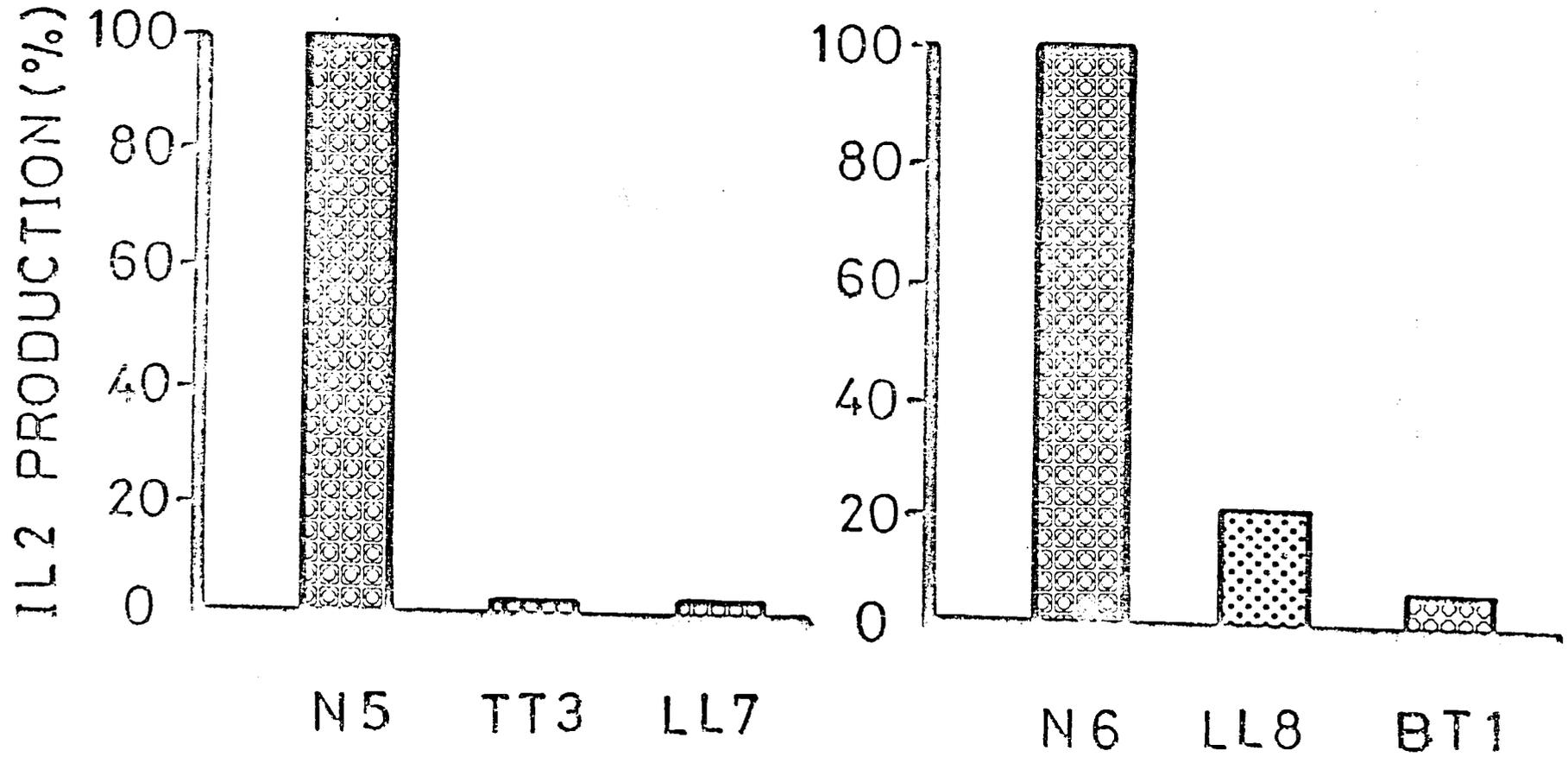


Figure 21. Production of interleukin 2 by PBML of normal subjects (N5 and N6), tuberculoid (TT3), borderline tuberculoid (BT1) and lepromatous leprosy (LL7 and LL8).

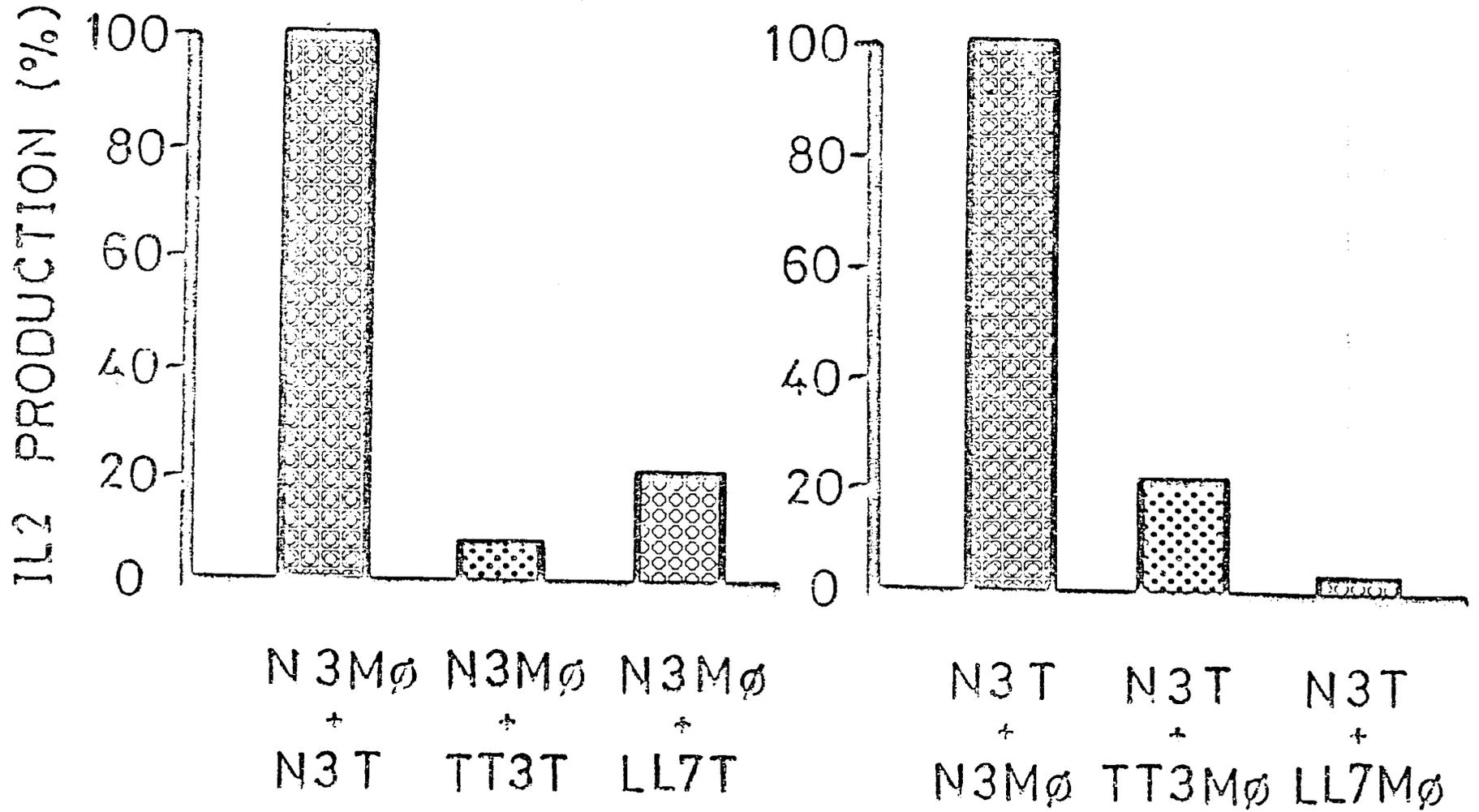


Figure 22. Production of interleukin 2 by mixed cultures of monocytes with homologous or heterologous T lymphocytes or vice versa (N = normal; Mø = monocytes; TT T = T lymphocytes from TT; LL T = T lymphocytes from LL).

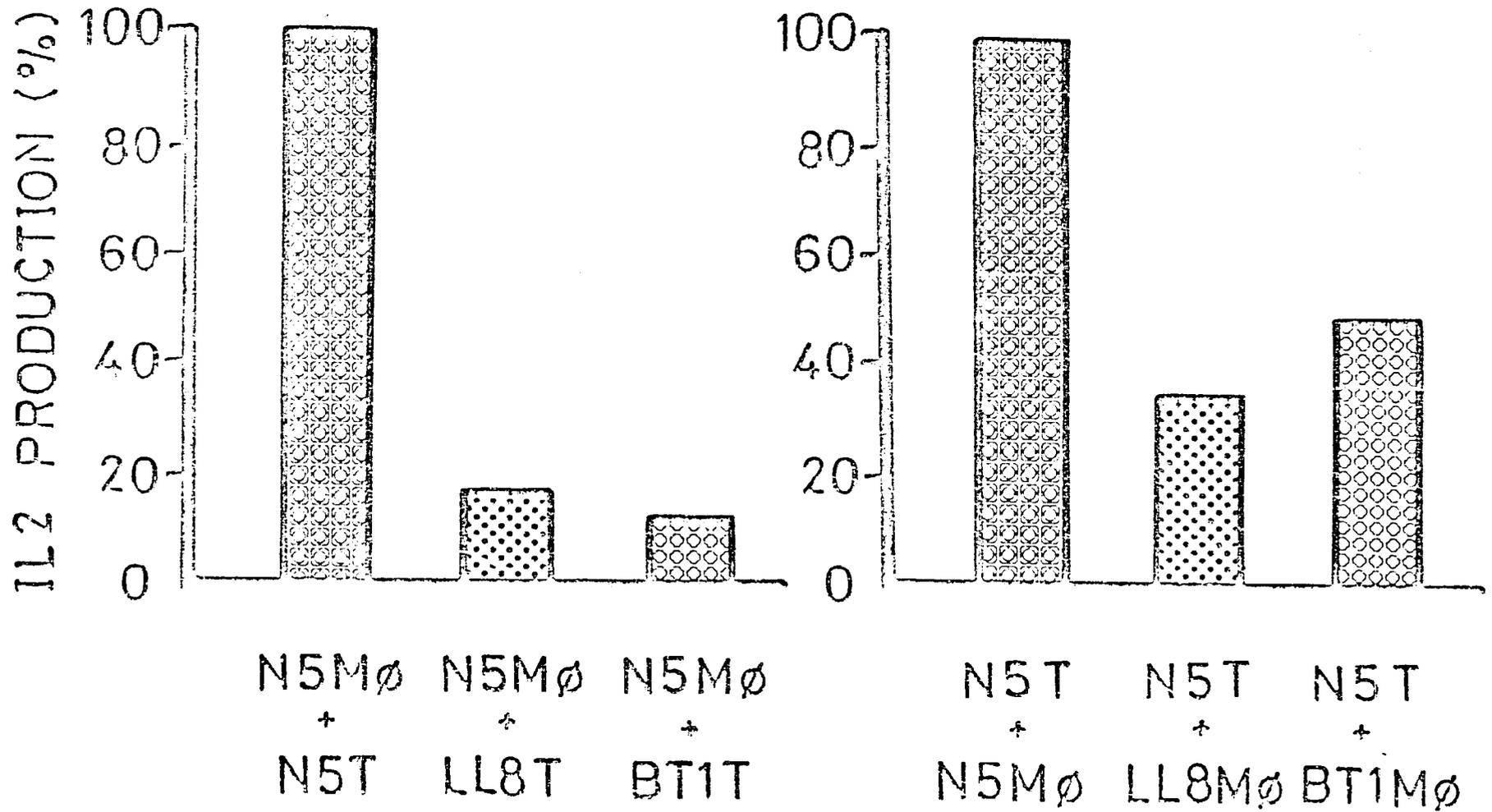


Figure 23. Production of interleukin 2 by mixed cultures of T lymphocytes with homologous or heterologous monocytes or vice versa (N = normal; T = T lymphocytes; TT Mø = monocytes from TT; LL Mø = monocytes from LL).