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Effect of C-Reactive Protein, PHA, PWM and Choline Phosphate in 3H-Thymidine Uptake of Leukocytes of Leprosy Patients and Normal Individuals[1, 2]

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Autologous serum used in lymphocyte cultures in transformation studies for the examination of cell-mediated immunity in leprosy patients has been shown to contain substance(s) which depressed the 3H-thymidine incorporation into DNA in phytohemagglutinin (PHA) stimulation of leukocytes ([4, 27]). Initial attempts at physical and chemical characterization of the serum factor have been made by Bullock and Fasal ([4]). In similar investigations in other diseases and in animal experiments a polypeptide fraction and extracts of spleen cells have been shown to inhibit 3H-thymidine uptake by lymphocytes in short term tissue cultures ([17, 20, 26]). Depression of 3H-thymidine incorporation into DNA of lymphocytes stimulated by PHA has been demonstrated in sera of patients with cancer ([2, 6, 17, 25, 31]), with trauma following surgery ([24]), in pregnancy ([18]) and with other diseases ([21]). The nature of these serum factor(s) with the possible exception of 17-hydroxycorticosteroids ([11, 28]) generally remains an enigma and has not been extensively investigated.

Recent studies from our laboratory have suggested that CRP, a common denominator in the sera of many disease processes ([3, 19]) cited below can depress the uptake of 3H-thymidine into the DNA of leukocytes ([14, 23]). This depression is reversible by the addition of choline phosphate to the culture system ([14, 23]). Choline phosphate was shown earlier by Volanakis and Kaplan ([30]) to bind readily to C-reactive protein (CRP) in the presence of Ca^{++}.

In this study the effect of autologous serum in lepromatous and tuberculoid leprosy patients was examined in 3H-thymidine uptake experiments with PHA and pokeweed mitogen (PWM) in the presence and absence of choline phosphate. In addition, the effect of purified γ-CRP on normal leukocyte incorporation of 3H-thymidine was examined and the reversibility of the effects with choline phosphate demonstrated.

The data presented are comparable to our previous study with PHA ([14, 23]) and suggest that in leprosy patients also, CRP in autologous serum may in part be associated with the depression of 3H-thymidine incorporation into the DNA of leukocytes.

MATERIALS AND METHODS

Selection of patients. Ten patients with active lepromatous, six with inactive lepromatous and five with active tuberculoid leprosy volunteered for this study. Blood samples from active lepromatous and active tuberculoid patients were obtained from the State Hospital, Hale Mohalu, Pearl City, Hawaii and Straub Clinic, Honolulu, Hawaii, while blood samples from the inactive lepromatous patients were obtained from the State Hospital, Kalaupapa Settlement on Molokai and from Dr. Caver, Honolulu, Hawaii. The ages ranged from 15 to 67 years in the active lepromatous and 38 to 67 in the inactive lepromatous category. In the active

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tuberculoid group the ages ranged from 14 to 45 years. The male:female ratios were 7:3 for active lepromatous, 5:1 for inactive lepromatous and 3:2 for the tuberculoid group. The patients, residents and immigrants from the Asian and Pacific basin, represented ethnic groups characteristic of the State of Hawaii. Active lepromatous or tuberculoid leprosy was confirmed by clinical examination, skin biopsy, skin smear for acid-fast bacillary count and, when available, lepromin tests. The individuals in the inactive lepromatous category at the time of blood sampling showed repeated negative skin smears at least for one year with no clinical signs of the active disease.

Leukocytes from 15 healthy individuals with undetectable CRP in their sera were examined as the control group. The ages ranged from 18 to 69 years and the male:female ratio was 9:6. The control group reflected the ethnic distribution of this state and was similar to that of the leprosy categories.

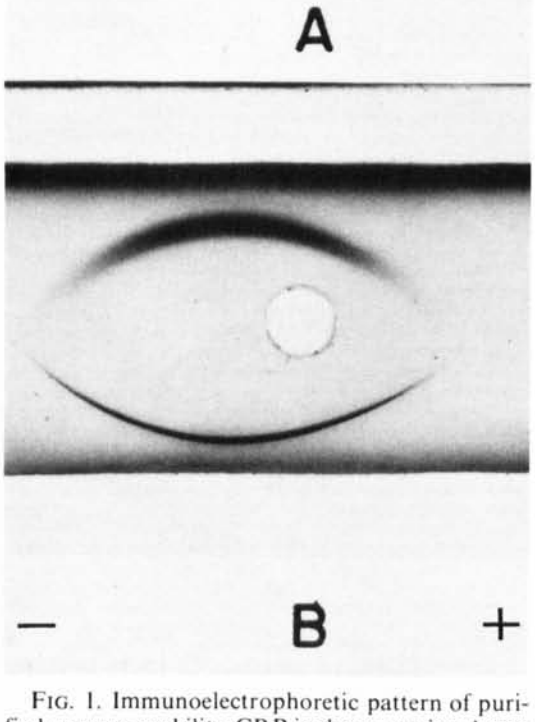

FIG. 1. Immunoelectrophoretic pattern of purified gamma-mobility CRP is shown against horse Anti-CRP, well A, and sheep Anti-CRP, well B, antisera.

C-reactive protein isolation and analysis. C-reactive protein assay was carried out by radial immunodiffusion as described elsewhere ([16]) using sheep anti-CRP (SCRPA).[4] C-reactive protein with gamma-mobility as illustrated in Figure 1 was isolated by gel filtration and DEAE-cellulose chromatography according to procedures of Hokama *et al* ([13, 15]).

Tissue culture for examination of PHA effect. The tissue culture system consisted of the following constituents: 0.02 ml tissue culture medium (TC); 0.10 ml untreated autologous plasma; 0.10 ml leukocytes (0.5 to 1.0×10^6 cells) suspended in autologous plasma; 0.10 ml Difco PHA-M diluted 1:3 with deionized sterile distilled water and 0.10 ml deionized water to give a final volume of 0.60 ml. Controls contained no PHA and the volume difference was compensated by the addition of sterile distilled water. The mixture was prepared in 25 × 75 mm sterile Falcon plastic tubes.

The TC medium consisted of 10.0% Basal medium-Eagle (BME)-Hank's, 10.0% fetal calf serum,[5] 1.0% glutamine, streptomycin and penicillin at 100 μ/ml each and the pH adjusted to 7.4 with sterile 7.5% sodium bicarbonate solution. Leukocytes were obtained from plasma and the buffy coat layer of freshly drawn heparinized blood samples and resuspended in autologous plasma (5.0 to 10.0×10^6 cell/ml).

The culture mixtures and appropriate controls were incubated for four days at 37°C without disturbing the cells. The 3H-thymidine containing 2.0 microcurie (specific activity 1.0 C/mM, International Chemical and Nuclear Corp. Irvine, California) in 0.05 ml sterile distilled water was added to each tube at the end of four days of culture. The cells in the tubes were mixed thoroughly and reincubated for four hours after which 0.1 ml of cold, nonradioactive thymidine (1000×) was added to each tube. The tubes were then shaken thoroughly to insure uniform suspension of the cells and 0.1 ml removed from each tube with a micropipet and placed on a 2.3 cm diameter Whatman 3MM filter paper circle.

[4]Sheep antiserum from Hawaii Immunological and Biological Laboratory, Kailua, Hawaii.

[5]BME-Hank's and fetal calf serum from Grand Island Biological Company, Grand Island, New York.

Radioactivity determination. The discs containing the samples were air-dried, placed in a 500 ml flask and washed with three successive aliquots of 250 ml 5% cold trichloracetic acid solution at 30 minute intervals. The paper discs were then suspended in 200 ml of 95% ethyl alcohol followed by subsequent washes in a 50% ethyl ether-alcohol mixture and anhydrous ethyl ether. The filter discs were air-dried and placed in counting vials. To each vial 10 ml of scintillation solution was added. The scintillation solution consisted of 5.0 gm 2,5-diphenyloxazole (PPO) and 0.5 gm of 1,4-bis-(2-[4-methyl-5-phenoloxazolyl]) -benzene (M_2POPOP) dissolved in one liter of toluene.[6]

The samples were counted for ten minutes at 4°C in a Packard Tri-Carb Scintillation Spectrometer. Following background correction the ratios of the counts between experimental and control values were determined. Cell data compiled are ratios of experimental divided by the control culture tubes. All samples were cultured in duplicate and radioactivity counts were carried out in duplicate for each culture tube. Thus, the ratios were obtained from the averages of four counts from duplicate cultures. Statistical analysis was done by methods described in the monograph by Mack ([22]).

Tissue culture with choline phosphate. The effect of the addition of choline phosphate at 500 μg/culture in 0.10 ml aliquot was examined in the presence and absence of PHA-M with respect to the 3H-thymidine uptake into DNA of leukocytes. This concentration was selected since earlier it was shown to give the best results ([14, 23]). These cultures were run in parallel for each preparation of leukocytes examined with the other studies.

Tissue culture with PWM. Corresponding culture tubes containing PWM were prepared with the same leukocyte suspension from each sample used in the previously described experiments. The TC system used was the same as that indicated for the PHA examination. PWM was diluted 1:10 with sterile deionized water and 0.10 ml was added to each tube. The effect of 500 μg/culture of choline phosphate on PWM stimulation was also examined.

Tissue culture of normal leukocytes with CRP. The effect of γ-mobility CRP on PHA-M stimulation of DNA synthesis of normal leukocytes was examined. CRP, 16 μg/culture, in 0.10 ml of 0.05 M phosphate buffer, pH 7.0, was added to PHA-M (0.10 ml of 1:3 dilution) in TC medium which contained leukocytes (0.5 to 1.0×10^6 cells) from healthy normal individuals. The effect of choline phosphate on the CRP PHA-M leukocyte culture was also examined by adding 500 μg/culture of choline phosphate to a similar set of culture tubes. The incorporation analysis was as described above.

RESULTS

Effect of choline phosphate on leukocytes simulated with PHA and PWM. Table 1 summarizes the effect of the addition of choline phosphate to the tissue culture systems of normal, active and inactive lepromatous and active tuberculoid individuals in the presence of PHA. Choline phosphate appeared to enhance significantly the incorporation of 3H-thymidine in all of the leprosy groups examined ($P<0.005$ to 0.050, column B of Table 1). Significant enhancement of 3H-thymidine uptake was also noted with normal leukocytes in the PHA response ($P<0.050$). With the exception of leukocytes of active tuberculoid leprosy (N.S.) significant differences in 3H-thymidine uptake were noted in the PHA study between normal leukocytes and the active and inactive lepromatous leukocytes, $P<0.001$ for the active group and $P<0.050$ for the inactive group (column A, Table 1). The active lepromatous group showed significant depression of 3H-thymidine uptake, while the inactive lepromatous group showed an increase in response to PHA over the normal group.

Table 2 summarized the ratio of 3H-thymidine uptake of leukocytes from normal individuals, active and inactive lepromatous and active tuberculoid leprosy patients in the presence of PWM. Addition of choline phosphate to the leukocyte-PWM cultures significantly enhanced 3H-thymidine uptake in all of the leprosy categories ($P<0.010$ to 0.050, column B). No significant increases were noted for normal leukocytes ($P>0.100$). Significant depression of 3H-thymidine uptake was noted for leukocytes of the active

[6]Scintillation solution compounds from Packard Instrument Co., Inc., Downers Grove, Illinois.

TABLE 1. *The effect of choline phosphate on the response of leukocytes of leprosy patients and normal individuals to PHA-M in 3H-thymidine incorporation.*

Leukocyte	Number of samples	Gamma-CRP (+/total)	Ratio of 3H-thymidine incorporation: Mean + S.D. PHA-M A	PHA-M + Choline-PO_4 B	Choline-PO_4 C
Normal	15	undetectable	7.51 ± 6.51	12.37 ± 8.15 $P < 0.050$	0.96 ± 0.09
Lepromatous active	10	10/10 (range:25-250 μg/ml)	1.54 ± 0.47 $P < 0.001$[a]	2.81 ± 1.65 $P < 0.005$	0.87 ± 0.35
Lepromatous inactive	6	2/6 (10.4 μg/ml)	10.75 ± 6.56 $P < 0.050$	17.45 ± 10.52 $P < 0.050$	1.13 ± 0.21
Tuberculoid active	6	1/6 (10.4 μg/ml)	5.95 ± 6.06 N.S.	9.40 ± 7.02 $P < 0.050$	0.95 ± 0.13

[a] Probability percentages are based on t-test ([22]): P values in column A represent comparisons of the means and variances of leprosy categories with the normal mean and its variance; while P values in column B are comparisons of the means and their variances within the same category, that is column A vs. B.

TABLE 2. *The effect of choline phosphate on the response of leukocytes of leprosy patients and normal individuals to PWM in 3H-thymidine incorporation.*

Leukocyte	Number of samples	Gamma-CRP (+/total)	Ratio of 3H-thymidine incorporation: Mean + S.D. PWM A	PWM + Choline-PO_4 B	Choline-PO_4 C
Normal	15	undetectable	4.43 ± 2.95	5.43 ± 3.78 $P > 0.100$	0.96 ± 0.90
Lepromatous active	10	10/10 (range: 25-250 μg/ml	2.31 ± 1.28 $P < 0.005$[a]	3.81 ± 2.16 $P < 0.010$	0.87 ± 0.35
Lepromatous inactive	6	2/6 (10.4 μg/ml)	2.94 ± 2.12 $P < 0.010$	9.19 ± 7.61 $P < 0.010$	1.13 ± 0.21
Tuberculoid active	5	1/6 (10.4 μg/ml)	5.79 ± 3.86 N.S.	8.61 ± 4.16 $P < 0.050$	0.95 ± 0.13

[a] Probability percentages are based on t-test ([22]): P values in column A represent comparisons of the means and variances of leprosy categories with the normal mean and its variances; while P values in column B are comparisons of the means and their variances within the same category, that is between columns A and B.

and inactive lepromatous groups ($P < 0.005$ and $P < 0.010$, respectively), while leukocytes of active tuberculoid leprosy showed no significant difference from normal leukocytes. These values are shown in column A.

Choline phosphate alone (500 μg/culture) showed neither significant enhancement nor suppression of 3H-thymidine incorporation under the conditions examined. These results are shown in both Tables 1 and 2, column C.

The CRP data for each disease category and of the normal controls are also shown in Tables 1 and 2. All of the active lepromatous cases gave positive γ-CRP and the levels ranged from 25 to 250 μg/ml of plasma. Two of six cases were positive in the inactive lepromatous group at 10.4 μg/ml of plasma, while one of five was positive in the tuberculoid group at 10.4 μg/ml of plasma.

The unstimulated control baseline counts/min/culture for the normal leukocytes ranged from 870 to 4060 with a mean of 1705; the active lepromatous ranged from 2640 to 4960 with a mean of 3710; the inactive lepromatous from 1280 to 3320 with a mean of 2070 and the active tuberculoid from 1030 to 1930 with a mean of 1460.

TABLE 3. *The relationship of CRP levels and the effect of addition of 500 μg of choline phosphate in the PHA response of leukocytes from lepromatous active leprosy patients.*

Patient no.	μg CRP/ml of plasma	Ratio of 3H-thymidine uptake: PHA-M	Ratio of 3H-thymidine uptake: PHA-M + Choline-PO_4
L-1	25.0	1.43	2.18
2	25.0	1.71	4.33
3	25.0	2.10	2.70
4	50.0	1.40	2.10
5	125.0	1.70	2.50
6	250.0	1.13	1.06
7	25.0	2.42	2.84
8	250.0	1.36	2.43
9	50.0	1.30	1.26
10	25.0	0.80	6.74
Total 10	range 25-250	1.54 ± 0.47	2.81 ± 1.65
		$P < 0.005$	

TABLE 4. *The effect of addition of purified C-reactive protein on the response of leukocytes from normal individuals to PHA-M in 3H-thymidine incorporation.*

Leukocyte	Number of samples	Ratio of 3H-thymidine incorporation: Mean + S.D. PHA-M A	PHA-M + CRP B	PHA-M + CRP + Choline-PO_4 C	Choline-PO_4 D
Normal	9	7.34 ± 6.10	3.78 ± 2.23	5.69 ± 2.00	1.08 ± 0.13
		$P < 0.010$[a]	$P < 0.010$		

[a]Percentage probability levels are based on t-test ([22]): P values represent comparison between columns A and B and columns B and C, respectively.

Comparison of CRP levels to PHA response of leukocytes of lepromatous active patients. The relationship of plasma CRP levels of active lepromatous patients to leukocyte responses to PHA in presence and absence of 500 μg of choline phosphate/culture tube is indicated in Table 3. In eight of the ten cases addition of choline phosphate showed an increase in 3H-thymidine uptake. The calculated levels of CRP added per culture based on the amount of autologous plasma used ranged from 10.0 to 100.0 μg/ml of medium. These levels of CRP have been shown to be inhibitory in our previous studies ([14, 23]).

Effect of gamma-CRP on normal leukocytes and the reversibility with choline phosphate. If CRP is involved, in part, in the depression of leukocyte incorporation of 3H-thymidine, then the addition of CRP to normal leukocyte cultures should show inhibition of 3H-thymidine uptake. This inhibition in turn should be reversed by the addition of choline phosphate. Results of such an examination from leukocytes of nine normal healthy individuals are shown in Table 4. Addition of 16 μg/culture tube of purified γ-CRP showed a significant depression of the 3H-thymidine uptake ($P < 0.010$). This inhibition was reversed by the addition of 500 μg/culture of choline phosphate ($P < 0.010$). Choline phosphate alone again showed essentially no stimulation or toxicity to the leukocytes. The homogeneity of the γ-CRP used in this study is demonstrated in Figure 1, which shows a typical γ-mobility CRP pattern reacting against SCRPA and HCRPA (horse anti-CRP) in immunoelectrophoretic analysis.

DISCUSSION

Independent of the choline phosphate effect, the inability of leukocytes of active lepromatous leprosy patients to respond ac-

tively to PHA stimulation by 3H-thymidine incorporation into DNA has been demonstrated. On the other hand, leukocytes of inactive lepromatous patients who had shown no signs of the disease for one year, showed a greater response to PHA than those of the normal control group. The results with the leukocytes of the active tuberculoid group were similar to those of the control group. These leukocytic responses to PHA in DNA synthesis are generally in agreement with previously reported studies of Bullock and Fasal [4], Sheagren *et al* [27], Dierks and Shepard [5], Han *et al* [8], and Wong *et al* [32].

The results are also in accord with generalized deficiencies attributable to cell-mediated immunity such as depressed lymphotoxin production [9] and survival of skin allografts in leprosy patients [7] and the impairment of lymphocyte transfer reactions in lepromatous patients [10]. Serum factor(s) such as CRP may be involved in the suppression in lepromatous patients in the latter study.

It is interesting to note that the mean baseline cpm/culture for the lepromatous active group (3710 cpm) was 2.16 times that of the normal mean value (1705 cpm). These higher baseline counts contributed, in part, to the lower ratio values in the active lepromatous patient group in both the PHA and PWM responses. This baseline stimulation may be caused by factor(s) in the autologous plasma or may be partly attributable to CRP which has been shown by immunofluorescent analysis to bind to lymphocytes [14].

With the exception of the active tuberculoid group, the leukocyte responses to PWM showed less DNA synthesis than those stimulated with PHA. This difference may have resulted from the selective stimulation of B-cells (thymus-independent) by PWM and stimulation of T-cells (thymus-dependent) by PHA as suggested by several investigators [1, 12]. Again, as in the PHA study, the depression of the PWM response was greatest with leukocytes of the active lepromatous patients.

Examination of 3H-thymidine uptake in the DNA synthesis of leukocytes with the addition of choline phosphate provided the most interesting findings. Evidently, both in normal as well as in all of the leprosy categories examined, serum factor(s) showing depression of 3H-thymidine uptake were observed. As shown earlier [14, 23], and in this study, choline phosphate significantly reversed the depressive effect of serum factor(s) in the PHA as well as the PWM culture systems. Gamma-CRP was detectable primarily in the active lepromatous cases which would indicate the presences of higher levels of CRP and hence neutralization by choline phosphate would give significant increases in DNA synthesis. This is shown in Tables 3 and 4. The higher incidences of CRP in active lepromatous cases are in accord with a previous observation [29].

It is interesting to note that choline phosphate alone shows no active enhancement of leukocytic responses to 3H-thymidine uptake into DNA. Whether choline phosphate reverses the CRP effect by complexing with it in the autologous plasma, as suggested in our previous reports [14, 23] remains to be examined. At this juncture the divergent effects of mixtures of high levels of CRP and of choline phosphate in the 3H-thymidine incorporation in leukocyte DNA synthesis are still unknown and are being investigated. Nonetheless, the use of choline phosphate, which appears to be essentially nontoxic alone, is strongly recommended when using autologous plasma in the examination of cell-mediated immune responses. This should enable one to differentiate lymphocytes with intrinsic cell-mediated deficiency from lymphocytes affected by nonspecific serum inhibitors, such as high levels of CRP in pathologic serum.

SUMMARY

The effect of phytohemagglutinin (PHA), pokeweed mitogen (PWM), C-reactive protein (CRP) and choline phosphate (choline-PO_4) on DNA synthesis using 3H-thymidine was measured in leukocytes from active and inactive lepromatous, active tuberculoid leprosy and normal individuals. In cultures containing autologous plasma the responses of active lepromatous leukocytes to PHA and PWM were significantly lower than the normal group. The responses of active tuberculoid leukocytes to PHA were essentially similar to that of the normal control group, while the leukocytes of inactive lepromatous showed a significant increase over the control group. The leukocytic responses of inactive lepromatous to PWM were significantly lower than those of the control and active tuberculoid categories. In all groups,

leukocyte responses to PHA and PWM were increased significantly with the addition of 500 μg of choline-PO_4, the exception being the control group to PWM. Choline phosphate alone had no effect on the 3H-thymidine uptake by leukocytes. Addition of γ-CRP to normal leukocytes depressed significantly 3H-thymidine incorporation and this depression was reversed by the subsequent addition of choline phosphate. Gamma-CRP was detected in all of the active lepromatous sera examined, while two of six and one of five were detected in the inactive lepromatous and active tuberculoid, respectively. It is suggested that CRP in autologous serum may, in part, be responsible for the depression of 3H-thymidine incorporation into DNA. Furthermore, the use of choline-PO_4 is suggested for cell-mediated immune studies where autologous plasma is used as part of the culture system.

RESUMEN

Se midió el efecto de la fitohemaglutinina (FHA), de la fitolaca (FL), de la proteina C-reactiva (PCR) y del fosfato de colina (colina-PO_4), en la síntesis de ADN, utilizando timidina-3H, en leucocitos de lepromatosos activos e inactivos, tuberculoides activos e individuos normales. En cultivos con plasma autólogo, las respuestas de los leucocitos de lepromatosos activos a FHA y FL fueron significativamente menores que en el grupo normal. Las respuestas de los leucocitos de los tuberculoides activos a la FHA fueron esencialmente similares a las del grupo control normal, mientras que los leucocitos de los lepromatosos inactivos mostraron un aumento significativo sobre el grupo control. Las respuestas leucocíticas de los lepromatosos inactivos a la FL fueron significativamente menores que las de los controles y los tuberculoides activos. En todos los grupos las respuestas leucocíticas a la FHA y a la FL aumentaron significativamente al añadir 500 μg de colina-PO_4, siendo la excepción el grupo control para FL. El fosfato de colina por si sólo no tuvo efecto sobre la incorporación de timidina-3H por los leucocitos. Cuando se añadió PCR gamma a los leucocitos normales se deprimió la incorporación de timidina-3H significativamente y esta depresión se invirtió cuando posteriormente se añadió fosfato de colina. Se detectó PCR-gamma en todos los sueros de lepromatosos activos examinados, mientras que sólo se detectó en 2 de 6 y en 1 de 5 de los lepromatosos inactivos y los de tuberculoides activos, respectivamente. Se sugiere que la PCR en el suero autólogo puede ser parcialmente responsable de la depresión de la incorporación de timidina-3H al ADN. Aún más, se sugiere utilizar colina-PO_4 para estudios de hipersensibilidad retardada o inmunidad celular en los cuales se utilize plasma autólogo como parte del sistema de cultivo.

RÉSUMÉ

Dans des leucocytes récoltés à partir de lèpre lépromateuse active ou inactive, de lèpre tuberculoïde active, et d'individus normaux, on a mesuré l'action de la phytohémmagglutinine (PHA), du mitogène obtenu à partir de raisin d'Amerique (PWM), de la protéine C-réactive (CRP), et de la phosphate choline (choline-PO_4) sur la synthèse de l'ADN. Pour ce faire, on a utilisé de la thymidine-3H. Dans des cultures contenant un plasma autologue, la réponse des leucocytes provenant de lèpre lépromateuse active envers la phytohémmagglutinine ou le mitogène PWM, était significativement plus faible que les réponses enregistrées sur des leucocytes provenant du groupe normal. La réponse au PHA des leucocytes provenant de lèpre tuberculoïde active, était essentiellement semblable à celle constatée dans le groupe témoin normal. Par contre les leucocytes de lèpre lépromateuse inactive présentaient une augmentation significative par rapport au groupe témoin. La réponse leucocytaire au PWM des lépromateux inactifs était significativement plus faible que celle des catégories—témoins ou tuberculoïdes actives. Dans tous les groupes, la réponse leucocytaire à la PHA et au PWM était significativement augmentée lorsqu'on ajoutait 500 μg de choline-PO_4, une exception étant cependant le groupe—témoin à l'égard de PWM. La phosphate choline seule n'avait pas d'action sur la captation de thymidine-3H par les leucocytes. L'addition de γ-CRP à des leucocytes normaux, a significativement diminué l'incorporation de thymidine-3H; cette diminution était supprimée lorsqu'on ajoutait ensuite de la phosphate choline. Dans tous les échantillons de sérum provenant de lèpre lépromateuse active qui ont été examinés, on a pu détecte de la γ-CRP, alors qu'une telle constatation n'a été faite que dans 2 serums inactifs sur 6, et dans 1 serum tuberculoïde actif sur 5. On suggère dès lors que la CRP dans le sérum autologue peut être partiellement responsable pour la diminution de l'incorporation de la thymidine-3H dans l'ADN. De plus on suggère d'utiliser la phosphate choline dans les études de l'immunité transmise par les cellules, lorsque du plasma autologue est employé dans le système de culture.

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