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SARCOID GRANULOMAS AFTER INTRADERMAL TUBERCULIN IN NORMAL HUMAN SKIN [1, 2]

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Over the years, a number of reports have appeared in the literature concerning the occasional development of persistent epithelioid granulomas at tuberculin skin test sites ([1-3]). These granulomas were delayed in appearance, usually at two to four weeks after the injection, and have followed the use of the purified protein derivative (PPD) of the tubercle bacillus as well as old tuberculin (OT). Histologically, these reactions have been described as "sarcoid," "tuberculoid," or actually resembling the granulomas of tuberculosis. No correlation between the development of this granulomatous change and the presence of active tuberculosis or other granulomatous disease could be made, however, and the reaction seemed to appear as readily in normals as in tuberculous patients.

There has been no satisfactory explanation of the pathogenesis of this granulomatous change. Contamination of the tuberculin test material and excessive tissue destruction, secondary to intense inflammation resulting from the tuberculin (48-hour) response, have been considered. Pascher, Sulzberger, and Satenstein invoked allergy in their interpretation of this reaction, although the precise mechanism was not made clear ([2]). In general, they related this peculiar granulomatous response to the 48-hour tuberculin allergy.

In the following report we have described our studies of the persistent epithelioid granulomas that develop in certain individuals following intradermal injection of PPD. As a result of these studies we have concluded that these granulomas are not due to contamination of the test material or secondary to tissue destruction, but may well be

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allergic granulomas whose pathogenesis is comparable to that of zirconium granulomas described below.

In 1957 we demonstrated that sarcoid granulomas could be produced in the human skin of susceptible individuals as a result of a delayed allergic hypersensitivity reaction to the metal zirconium (4). Originally sensitized through the use of zirconium-containing deodorants, these patients would respond with a local sarcoid granuloma to skin test doses of as little as 0.2 μg. of any water-soluble zirconium salt. Characteristically, no significant change was seen at the test sites at 48-72 hours, but within three to five weeks a granulomatous reaction developed and would persist for a few months to a year or more. It is to be stressed that these patients were otherwise normal and healthy, without evidence of any other granulomatous disease and with no correlation of this reaction with tuberculin skin test reactivity. Moreover, they did not react abnormally or distinctively to nonallergic granulomagenic stimuli, such as sodium stearate (5). Control injections of other metals and inorganic elements in these zirconium-sensitive patients, as well as in many normal healthy volunteers, were negative. All of these data verified the specificity of this distinctive allergic response. This was the first demonstration *in man* that allergic hypersensitivity to a pure chemical could be responsible for the development of a granulomatous change.

In addition to the observations on zirconium granulomas, the literature contains other evidence indicating the existence of a special type of allergy which results in epithelioid granulomas. In recent years it has been demonstrated that in animals sensitized to a foreign protein the subsequent injection of that protein may give rise to extensive granulomatous changes (6, 7). In some instances, specific antibody demonstration has apparently been achieved as well (6). Sneddon elicited delayed local granulomas in patients with berylliosis, three weeks after patch testing with 1 per cent beryllium sulfate (8). Although no intradermal tests with appropriate dilutions of beryllium salts were done, we would view the patch-test granulomas in such patients as an indication of their hypersensitivity to beryllium which manifested itself as a sarcoid granuloma. The patch test duplicated the histopathologic pattern characteristic of the disease, berylliosis.

In patients with sarcoidosis, the intradermal injection of a 10% saline extract of sarcoidosis tissue, usually lymph node or spleen, results in the development of a local sarcoid granuloma at three to five weeks (9) No change is evident at the test site before this time. Unfortunately, this test (Kveim test) has often lead to erratic and negative results, probably because preparation and standardization of the antigen are difficult. Actually, the granulomagenic agent within the test preparations (Kveim antigen) has not yet been identified.

In tuberculoid leprosy, but not in the lepromatous form of the

disease, the intradermal injection of a bacillus-rich suspension of the whole leproma (lepromin) will result after three to five weeks in the production of a granuloma (the Mitsuda reaction) typically more or less of sarcoid structure ([10]).Usually in such cases, but not invariably, there is also a tuberculin-like response after 24-48 hours (the Fernandez reaction). Large proportions of normal persons, never exposed to leprosy and tuberculin negative, will show some degree of the same late (Mitsuda) reaction, usually without having shown the earlier reaction. Hence, as found in our study with tuberculin, the granulomatous lesion does not necessarily depend on the effects of the earlier inflammatory reaction. Among leprosy patients, it is thought that those with the tuberculoid form are specially prepared immunologically to react in this distinctive manner whereas lepromatous cases are specifically nonreactive; the late (Mitsuda) reaction in normals is held by some workers to result from immunologic changes induced by the injected antigen (the "microvaccination" of Ustvedt). Filtrates of the lepromin suspensions, and protein extracts of the bacilli, give only the Fernandez reaction, which is ascribed to a pre-existing tuberculin-like hypersensitivity. The Mitsuda response occurs only after injection of the bacillary bodies, and is proportionately weakened as the bacilli are broken down by grinding or sonic treatment, or by extraction of the lipid elements by solvents. It is of special interest that the Mitsuda reaction is not greatly accelerated—when at all—in leprosy-infected reactors over the reaction time in positively reacting normals.

The fundamental similarity of the skin test reactions in berylliosis, sarcoidosis, tuberculoid leprosy, and zirconium granulomatoses supports the thesis that a special form of granulomatous hypersensitivity exists and is operative in the pathogenesis of these diseases.

METHODS AND MATERIALS

Fifty normal, healthy Negro male volunteers, ranging in age from 22 to 60 years, were employed in this study. Commercially available PPD of the tubercle bacillus was used throughout these experiments. The diluent employed was sterile isotonic saline, not that which is routinely supplied with the PPD. Skin tests were done on the forearm and the dose given was 0.05 cc., in contrast to the 0.1 cc. normally employed in routine tuberculin skin testing. Control injections of 0.05 cc. of the isotonic saline were given routinely on the opposite forearm. The skin test sites were observed at 48 hours and then weekly for four to five weeks. The 48-hour reactions were graded roughly as +1, +2, +3, or +4, according to the intensity of the response. Clinical changes, such as vesiculation or bulla formation, were noted also. The four-week reactions were graded similarly. Questionable or very faintly palpable reactions were recorded as negative. Biopsies of selected sites were taken at 48-72 hours and at four weeks. Tissue was stained with hematoxylin and eosin or by the Van Gieson technique after formalin fixation.

OBSERVATIONS

Incidence of granulomatous response to first strength PPD.—All 50 men were tested with first strength PPD initially. The control in-

jection sites were devoid of clinical change throughout the entire four-week observation period. There was a reasonable degree of variation in the intensity of the 48-hour tuberculin responses. Fourteen of the subjects failed to react to this strength tuberculin. Five of the subjects demonstrated a painless papule persisting through four weeks. Biopsies of these papules revealed focal epithelioid granulomatous change in each. Central necrosis was absent in these granulomas, and the number of associated inflammatory cells varied among the five subjects. In two of the men very few round cells were visualized (Fig. 1). In the other three, however, a moderate number of chronic inflammatory cells were noted, often arranged peripheral to the epithelioid cells (Fig. 2).

Additional skin tests with first strength PPD were done in four of these five subjects who had developed the granulomatous reactions. The fifth subject was unavailable for further study. Four additional tests were done in each subject. Two of these tests were done one week later, and two additional injections were given two weeks later. At 72 hours, the reactions were equivalent in intensity to those of the initial tuberculin tests. Biopsies of these sites revealed acute inflammatory changes, with edema of the corium, round-cell infiltration, principally perivascular, some spongiosis, and occasional tiny intraepidermal vesicle formation. No epithelioid cell change was evident at this time. At four weeks, firm papules again were noted at these sites in each of these subjects. Biopsy revealed epithelioid granulomas similar to what was described above. In two of the subjects the remaining PPD papules became impalpable within four and one-half to five weeks. In the other two, however, a palpable lesion was present at seven to eight weeks, although the lesions were resolving slowly. At six weeks, biopsy of one papule of one subject revealed a sarcoid granulomatous patterning, with very few inflammatory cells (Fig. 3). The number of inflammatory cells was fewer than at the four-week period. Biopsy at eight weeks of a faint residual papule, at a skin test site of the other subject, revealed a late sarcoid reaction with evidence of reticulum fiber formation. This is nicely illustrated in Figure 4. Over the course of the next six to nine months, the four subjects in whom the granulomas developed received three additional first-strength tuberculin tests to confirm the specificity of their distinctive reactivity. In each subject, reactions of a quality and intensity comparable to that which had been noted after the first tuberculin test, were seen.

In fifteen of the subjects who failed to develop a persistent papule, biopsy specimens of test sites at the four-week period were secured. These revealed only varying degrees of perivascular round-cell infiltration with no evidence of granulomatous change. Included in this group of fifteen subjects were those with clinically mild tuberculin reactions, as well as those with intense responses at 48 hours. It should be stressed that no definite correlation could be made between the intensity of the

48-hour tuberculin reaction and the development of granulomas at these test sites. Several subjects who manifested intense 48-hour tuberculin reactions failed to develop any persistent granuloma. Moreover, in the four subjects who did develop a local granuloma at the tuberculin test sites, the 72-hour response varied from +1 to +3. None of the subjects with negative 48-hour tuberculin reactions developed a delayed granulomatous change.

Effect of increased concentration of PPD.—In an effort to enhance or potentiate the granulomatous response to PPD, it was decided to examine the effect of increased concentration of PPD. Injections of PPD, 0.05 cc., in concentrations as indicated below, were given on the forearms of three of the four special subjects who had developed granulomas to first-strength PPD and to six control subjects who failed to show granulomatous change after tuberculin

1. First strength PPD (1:5,000,000)
2. Intermediate strength PPD (1:2,5000,000)
3. Second strength PPD (1:20,000)
4. Third strength PPD (1:10,000)

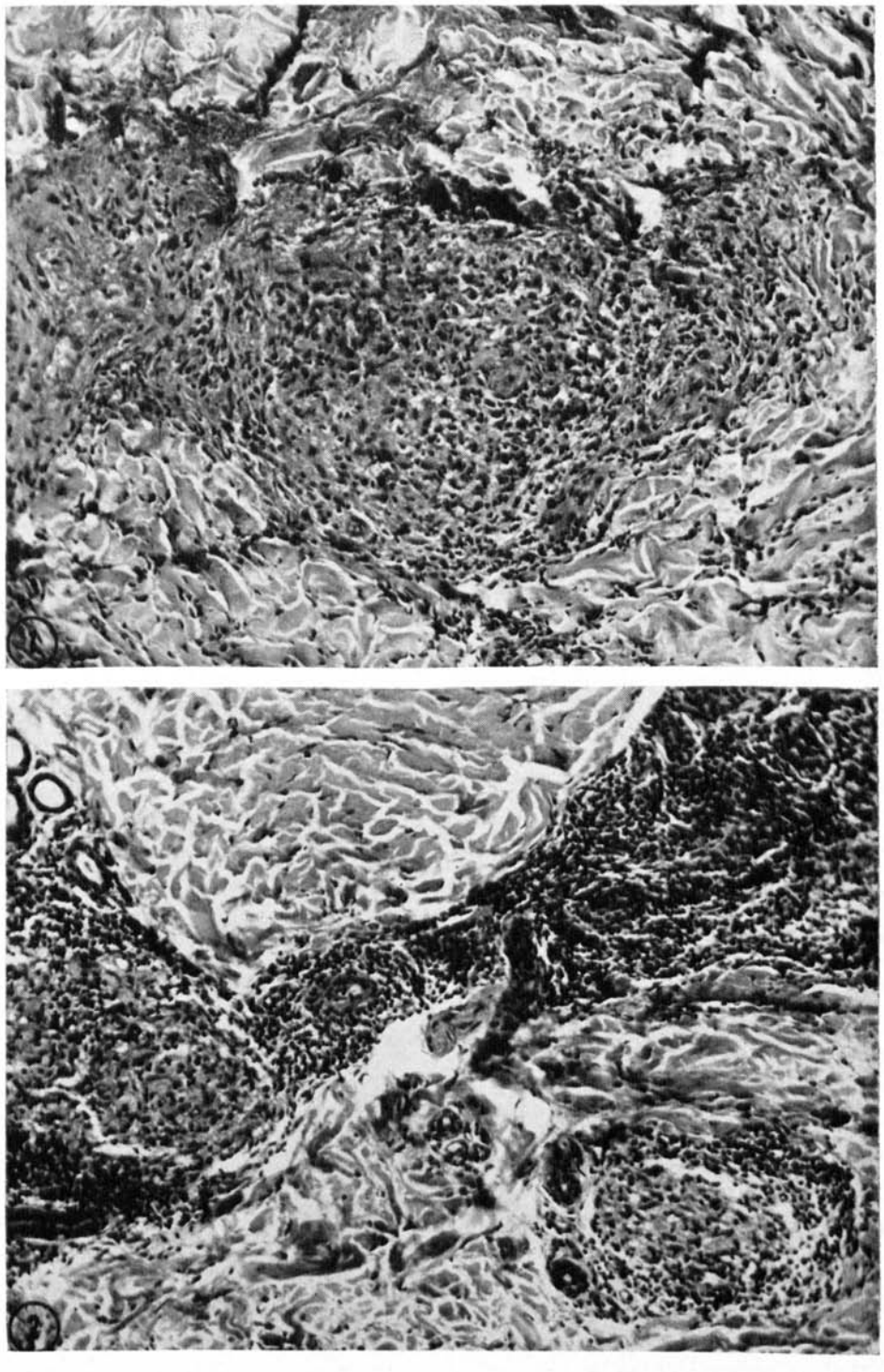
These injections were not all given simultaneously, but rather at two-week intervals. In only three subjects were we able to proceed with the third-strength tuberculin injection. Two of these were granuloma subjects and one was a control who had been negative to first-strength PPD, but had reacted to second-strength PPD.

In all of the subjects treated it was noted that the 48-hour responses were more intense with the increasing concentrations of tuberculin. However, persistent papules failed to develop in the six control subjects, regardless of the concentration employed and despite intense 48-hour reactions, including vesiculation, The three granuloma patients all again responded with local granulomatous reactions at each of the injection sites. Increasing the concentration resulted in more intense 48-hour responses in these subjects. In addition, the later (four-week) papules were larger with increasing concentrations of PPD. Histologically, some variation in size of the reactions was noted as well.

DESCRIPTION OF PLATE

FIG. 1. Sarcoid granuloma at four weeks after first strength PPD. 0.05 cc. first-strength PPD injected intradermally in the forearm produced a +1 erythematous papule at 48 hours. At four weeks a firm, painless papule was noted. On biopsy focal epithelioid granulomatous patterning was evident. Note the lack of central necrosis and minimum of associated inflammatory cells consistent with the diagnosis of sarcoid granuloma.

FIG. 2. Tuberculoid granuloma at four weeks after intradermal injection of first-strength PPD. The photograph represents the histological appearance of a papule which persisted through four weeks after the intradermal injection of first-strength PPD. Note the tuberculoid granulomatous patterning with a sheath of inflammatory cells surrounding the epithelioid cells. Compare with Figures 1 and 3. The overall pattern is fundamentally comparable except for the inflammatory cell infiltrate.



However, these quantitative differences were not remarkable considering the great increase in the concentration of the PPD. Furthermore, histologically, there were no fundamental qualitative differences in the type of reactions seen through all concentrations employed. For each subject the granulomatous change was constant for all of the concentrations. Sarcoid or tuberculin granulomatous patterning was visualized as in the earlier studies (Figs. 1-4). It was concluded that increasing the concentration of the PPD, within the limits allowed, did not of itself provoke granulomas in subjects in whom weaker concentrations failed to induce this change.

COMMENT

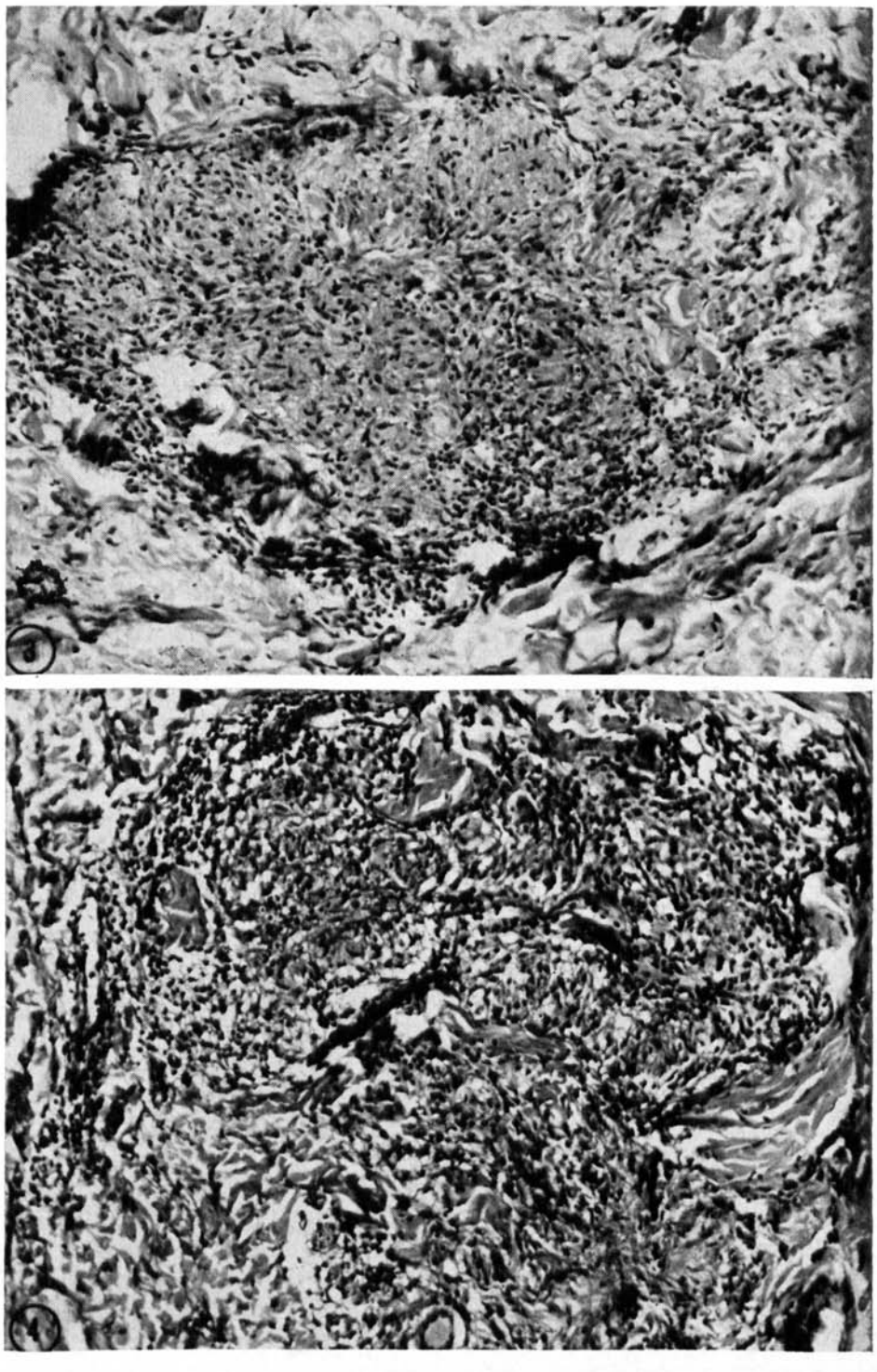
On the basis of these studies, it seems possible to separate the classical 48-hour tuberculin response from a delayed granulomatous reaction to the same substance: the purified protein derivative of the tubercle bacillus. This granulomatous change did not develop in everyone, but rather in a limited number of individuals, 10 per cent of our experimental group of 50 adult Negro males. It is to be stressed that these people were healthy, without evidence of active tuberculosis, sarcoidosis, or other disease. There was no correlation of the granulomatous reaction with that of the 48-hour response. Indeed, subjects with intense 48-hour tuberculin reactions failed to develop the granulomas. Furthermore, increasing the concentration of PPD in these individuals failed to provoke this granulomatous reaction, despite more intense 48-hour tuberculin reactions. This, plus the lack of extensive tissue necrosis histologically in the granulomatous lesions, supports the view that such granulomas were not secondary to intense inflammation and tissue destruction due to tuberculin (48-hour) reactions.

In this regard, it should be reiterated that these skin test injections were given intradermally and not subcutaneously. It has been known for some time that the subcutaneous injection of many substances, including tuberculin, may produce an intense inflammatory reaction with apparent release of lipids from the subcutaneous fat, and subsequent granuloma formation ([11]). It seems clear, also, that contamination of the PPD test material could not account for this reaction.

DESCRIPTION OF PLATE

FIG. 3. Sarcoid granuloma at six weeks after first-strength PPD. In one subject in whom, at the site of a first-strength PPD intradermal injection, a papule persisted through six weeks, biopsy revealed this sarcoid granuloma. Note the decreased number of inflammatory cells at this time as compared with earlier granuloma shown in Fig. 1.

FIG. 4. Late sarcoid reaction eight weeks after intradermal first-strength PPD. Eight weeks following 0.05 cc. of first strength PPD a small resolving papule was removed for histological study. This picture, with focal arrangement of nests of epithelioid cells, was seen. Presence of increased numbers of reticulum fibers indicating apparent involution of the granuloma is noted.



The control injections of the diluent were uniformly negative throughout all of these studies, and repeated testing with different samples of PPD failed to alter the response or lack of response in the various subjects. Finally, spectrographic analysis of the diluent for beryllium, xirconium, and silicon failed to reveal any significant quantities of these or other metals.

Accordingly, it is necessary to look for some other explanation for these granulomas. It is our view that they may well be the result of a special type of allergic hypersensitivity analogous to that of patients with zirconium and other sarcoid granulomas. In support of this is the delayed development of the reaction, as in other sarcoid granulomas, the limitation of the response to a few people, the reproducibility of the reaction in these individuals, and the fact that this reaction developed in response to as little as 0.000025 mg. of PPD. Increasing the concentration of PPD could conceivably call forth the granulomatous reactivity in other individuals, although we were unable to achieve this in the subjects tested, and the intensity of the 48-hour reactions prohibited any further increase in PPD concentration. In this regard, it should be recalled that the concentration of zirconium required to elicit the granulomatous response in susceptible patients was generally in the range of 1:10,000 (0.002 mg.), although some individuals reacted to weaker concentrations.

It is tempting to theorize on the possible importance of this granuloma hypersensitivity of PPD in the pathogenesis of the granulomas of active tuberculosis. Rich has stressed that this facet of the pathogenesis of tuberculosis has not been satisfactorily elucidated to date on the basis of release of lipids from the organisms themselves, or as a result of tissue injury ([12]). It is well to note, also, that PPD is but one of at least seven antigenic substances that have been isolated from the tubercle bacillus ([13]). Although their antigenic capacities vary, any or all of these antigens should be suspect in the analysis of the possible role of granuloma hypersensitivity in the pathogenesis of this disease. The fact that epithelioid granulomas have been described after BCG inoculations is of interest in this regard ([14]).

In recent years, it has become increasingly evident that the tubercle bacillus is of much less importance as a possible cause of sarcoidosis than was once thought. This despite a number of reports which seemed to relate this organism to sarcoidosis. The development of active tuberculosis in patients with sarcoidosis has been described on several occasions ([11, 15]). Conversely, the apparent progression from culture-proved tuberculosis to frank sarcoidosis has also been recorded ([11, 16]). A loss of tuberculin skin test reactivity has apparently accompanied this transition. In at least one report of such a case, a granulomatous papule developing at four to five weeks following an intradermal tuberculin test was described ([17]). The author concluded that his patient

had sarcoidosis due to the tubercle bacillus. On the basis of this one case he postulated further that all cases of sarcoidosis were related etiologically to the tubercle bacillus. A later study by Bjornstad negated the latter concept, since he demonstrated that such granulomatous reactions to tuberculin may occur in normals, as well as in patients with sarcoidosis ([3]).

In the light of this evidence it has been difficult to assign any special significance to the epithelioid granulomas which may develop after intradermal PPD. However, current thinking dictates that sarcoid granulomas represent not a single disease entity, but rather a reaction pattern which may be produced by any number of agents. As we indicated earlier, this reaction may be induced by a variety of subjects through the mechanism of a newly described type of hypersensitivity which manifests itself as a granuloma. It would seem judicious to re-evaluate the importance of the tubercle bacillus in the pathogenesis of sarcoidosis in terms of this new concept. It is unlikely, however, that the tubercle bacillus, or one of its antigenic components, would be a common cause of this disease.

SUMMARY AND CONCLUSIONS

The development of delayed (four weeks), palpable, epithelioid granulomas at sites of intradermal injections of first-strength tuberculin (PPD) is described. Such clinically evident granulomas were elicited in 5 of 50 healthy adult males so tested. Histologically, sarcoid or tuberculoid granulomatous patterning was visualized. It was stressed that these reactions were to a highly purified crystalline material, and no correlation could be made between the intensity of the 48-hour tuberculin response and the development of the granulomas. Further testing revealed that this reactivity was limited to these few men, and was consistently reproducible in each. Moreover, increasing the concentration of PPD did not induce granulomas in individuals in whom weaker concentrations had failed to stimulate such a reaction.

It was concluded that the development of these epithelioid granulomas could not be accounted for on the basis of tissue destruction or contamination of the PPD test material. It was postulated that allergic hypersensitivity could induce this granulomatous change in a manner analogous to that of the zirconium granulomas. The possible importance of this reactivity in the pathogenesis of tuberculoid granulomas and sarcoidosis is discussed.

RESUMEN Y CONCLUSIONES

Se describe la formación de granulomas epitelioideos, palpables, tardíos (cuatro semanas) en los sitios de inyecciones intradérmicas de tuberculina (DPP) de la primera concentración. Esos granulomas clínicamente evidentes se produjeron en 5 de 50 varones adultos sanos comprobados en dicha forma. Histológicamente, se visualizó un patrón sarcoideo o tuberculoideo. Se recalca que esas reacciones fueron a una sustancia

cristalina sumamente purificada, sin que pudiera establecerse ninguna correlación entre la respuesta a la tuberculina a las 48 horas y la aparición de los granulomas. Nuevas pruebas revelaron que dicha reactividad estaba limitada a esos pocos sujetos, y era constantemente reproducible en cada uno de ellos. Además, el aumento de la concentración del DPP no provocó granulomas en los individuos en quienes diluciones más débiles no habían excitado una reacción de ese gènero.

Se deducte que la formación de esos granulomas epitelioideos no puede explicarse a base de la histólisis o la contaminación de la materia de ensayo en el DPP. Se presupone que la hipersensibilidad alérgica podría inducir esta alteración granulomatosa en forma semejante a los que sucede en el granuloma zircónico. Discútese la posible importancia de esta reactividad en la patogenia de los granulomas tuberculoidoes y la sarcoidosis.

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