

Editorial

The Kveim response: still useful, still a puzzle

A skin test using sarcoid tissue to demonstrate cutaneous reactivity in individuals with probable sarcoidosis was described in 1935 by Williams and Nickerson,¹ but they did not report the histology of the responses. In 1941 Ansgar Kveim,² an Oslo dermatologist, published a complete description of the test that now bears his name. He noted that in subjects with sarcoidosis intradermal injection of a crude homogenate of sarcoid tissue produced, over several weeks, papules which on histological examination contained epithelioid cell granulomas exactly like those of the disorder. Reactions in control subjects, including some with tuberculosis, were negative. Subsequent investigators, notably Putkonen,³ Danbolt,⁴ and Nelson,^{5,6} used crude ("conventional") suspensions derived from various tissues to confirm the particular potency of sarcoid tissue in selectively inducing granulomas in subjects with sarcoidosis. The greatest individual contribution to the further study of the response was that of Louis Siltzbach, whose name is often appended to that of Kveim in the eponym. With Merrill Chase he was responsible for the development of the more finely particulate ("type I") suspensions used in current practice.^{7,8} Personally and in collaboration, Siltzbach also delineated in different forms of sarcoidosis the rate and pattern of positive responses, and their uniformity in more than 30 countries.^{9,10}

Nevertheless, the Kveim response remains a mystery. Nearly 50 years on, it might be expected that a skin test of such crude and arcane nature would have been superseded or at least refined; but, despite many advances in knowledge of the pathology and diagnosis of sarcoidosis, basic questions about aetiology and pathogenesis are unanswered. The purpose of this article is to review practical aspects of the use of the Kveim response and to consider, in the light of more recent and sophisticated approaches, its place both in the diagnosis of sarcoidosis and in the scientific study of granuloma formation.

Practical aspects, clinical use, and diagnostic significance

PREPARATION, VALIDATION, AND SAFETY OF TEST SUSPENSIONS

Lymphoid tissue affected by sarcoidosis is the most reliable potential source of Kveim material; of this, spleens removed for clinical reasons provide the largest amounts of material and hence the greatest continuity of supply. The organ is stored at -20°C or lower, and is thawed as required. To prepare a test suspension, the tissue is processed according to the method of Chase and Siltzbach.^{7,8} It is scissored, dispersed, and suspended in isotonic saline, washed twice by centrifugation at 5500 g, and strained through a succession of fine mesh filters. Routinely, the resultant suspension is pasteurised by heating three times to 58°C for one hour, and sterility is checked throughout. Phenolisation (0.25%) and in recent years the further exposure of the ampoules containing the suspension to 25 000 Gy (2.5 Mrad) from a cobalt-60 source (see below) complete the preparation. The particle concentration is up to 8 mg/ml, giving a test dose of 0.5–1.0 μg , but this may require adjustment in the light of clinical testing.

Validation of a Kveim suspension for diagnostic use can be achieved only by clinical testing; there is no identifiable feature of the source tissue or final suspension that guarantees its effectiveness. Initially, new suspensions can be tested alongside existing ones, and a concordance of 80–90% is desirable. Siltzbach¹¹ has defined criteria for full validation based on the performance of the best suspensions. Essentially, these are that the suspension should produce positive responses in 60% or more of cases of sarcoidosis of less than two years' known duration, and that in a third of the positive responses the papule should be 5 mm or more in diameter. Fewer than 3% of control subjects, including both healthy subjects and those with a range of diseases that may potentially be confused with sarcoidosis, should react positively. These properties of acceptable Kveim suspensions are referred to respectively as potency and selectivity for sarcoidosis. Fewer than half of the spleens acceptable for validation meet the criteria¹²; they may fail because of low potency or poor selectivity, or both. It is unwise to assume that sequential

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batches ("lots") prepared from different areas of the same spleen will have the same properties, and if this method of preparation is adopted a modified validation procedure should be undertaken for each new batch. If, alternatively, the whole spleen is processed initially there is a theoretical risk that a "rogue" portion might adversely affect the behaviour of the whole suspension. Nevertheless, because both preparation and validation are tedious and time consuming, suspensions now undergoing validation are representative of the whole of each splenic source. Several validated suspensions have been stored for long periods at room temperature or 4°C without loss of potency or selectivity,^{11 12} but possibly the storage properties of suspensions from different spleens vary. Stability might be better conserved by freeze drying, and Douglas¹³ and Kennedy¹⁴ showed that freeze dried material retained diagnostic potency after storage at room temperature for many months. It is doubtful, however, whether the time and expense of this form of preparation are justifiable at present. In practice, test materials are stored as suspensions at +4°C, and after release for clinical use their diagnostic performance is monitored.

In the past, the safety of the Kveim test has been questioned on theoretical grounds,¹⁵ and it is natural to view with concern the transfer from man to man of tissue from a disease of unknown aetiology. Because a single spleen is widely used, assessment of both the donor and the potentially useful tissue must be cautious. Fortunately, clinical circumstances permit more thorough investigation than in, for example, blood or organ donation. The use of serological tests for viral infection (including hepatitis B surface antigen, human immunodeficiency virus, and cytomegalovirus), heating during preparation of the suspensions, and microbiological and animal testing can be used to ensure the absence of recognised conventional pathogens; but the recent experience of slow virus transmission by human pituitary extracts emphasises the need for wariness. All Kveim material supplied for use in the United Kingdom is now irradiated (cobalt 60, 2.5 Mrad (25 000 Gy), a process that appears to have no important effect on diagnostic properties.¹⁶ In practice, the Kveim test appears to be free from adverse effects, save occasional local ulceration in strongly positive responses.

PERFORMANCE OF THE TEST AND ASSESSMENT OF RESULTS

Validated suspensions for diagnostic use in the UK are available in single dose ampoules from the Public Health Laboratory Service Centre for Applied Microbiology and Research (Vaccine Research and Production Laboratory, Porton Down, Salisbury, Wiltshire SP4 0JG). A charge of £10 a dose is made to

defray the expenses of preparation and dispatch. Elsewhere, availability of adequately validated suspensions is patchy; in the USA, federal regulation of interstate transport inhibits distribution of Kveim suspensions, although several centres produce them.

For injection, a 1 ml tuberculin type syringe with a narrow (26) gauge bevelled needle, through which the suspension will pass freely, is preferred. It is helpful to rinse the syringe and needle with sterile saline or water for injection to remove potential birefringent or other extraneous material. The suspension should be dispersed by shaking the ampoule well, and the test dose (0.15 ml) withdrawn. The usual site for testing is the medial aspect of the upper forearm, where any residual mark will be inconspicuous. Injection should be intradermal, raising a "peau d'orange" bleb, and not subcutaneous. It is also crucial to be able to identify the site for biopsy. Although this may be done by careful measurement in relation to landmarks such as moles, it is more practical to mark the site with a light tattoo of sterile Pelikan ink (just sufficient to break the epidermis) placed over the centre of the bleb.

Optimal results are obtained by examination 4–6 weeks after injection. A macroscopic assessment of response size in terms of erythema and induration should be recorded, at least on the form which accompanies the ampoule. In caucasians the typical papule of a positive response is often only some 3 mm in diameter. Large papules, 5 mm or more, are commoner among Afrocaribbean and Afroamerican subjects,¹⁷ and in any ethnic group are more likely to be histologically positive than smaller papules, just visible or barely palpable reactions, or clinically undetectable responses. But even the latter sometimes surprise and all responses, whether florid or undetectable clinically, should be routinely biopsied. Biopsy of all responses is obligatory in validation studies and failure to biopsy macroscopically negative sites will significantly vitiate results in clinical practice. Only local anaesthesia is necessary. Ellipse biopsy specimens are often taken, but 3 or 4 mm specimens taken with a Hayes Martin skin punch (Charles F Thackray) or its convenient disposable equivalent (Steifel) are simple, adequate and preferable, providing that the biopsy includes the whole dermis. Punch biopsies of very large papules should be slightly tangential so as to include the edge of the lesion, since the centre may show only collagen necrosis. It may be necessary to cut through subcutaneous adipose tissue with fine sterile scissors to release the specimen. The wound can be sutured, or closed with adhesive strips or (for punch biopsies) a single "butterfly" adhesive plaster.

For histological assessment, the specimen is fixed in neutral 10% formalin and processed conventionally. Ribbon-mounted serial 5–7 μm sections, of which

about every 20th is examined, from throughout the specimen give a good diagnostic yield. A detailed guide to histological interpretation is given by Scadding and Mitchell.¹² In many cases of sarcoidosis the test results in well formed epithelioid cell granulomas, mainly in the middle and deep dermis. The granulomas may be discrete or confluent, may be "naked" or may be cuffed by lymphocytic infiltrates. Central collagen necrosis is a feature of florid responses. However, interpretation of the histological findings can be difficult, for there is a no man's land of "equivocal" granulomatous responses which cause most of the observer variation in interpretation.^{18,19} Equivocal appearances constitute 10–20% of responses encountered in routine clinical practice, and include those in which epithelioid cells are diffusely scattered in the dermis, or in which granulomatous aggregates of histiocytes do not assume the circumscribed and organised foci of epithelioid granulomas. The presence of birefringent particles can make interpretation of such responses particularly difficult. Finally, while macroscopically negative responses usually contain only sparse evidence of dermal disruption, papular responses may occasionally be microscopically negative, containing only mixed infiltrates of lymphocytes and monocytes without any evidence of granuloma formation. In view of the variety of potential response, in particular the occasional equivocal granulomatous response, it is unfair to ask the pathologist always to provide the clear "positive" or "negative" which the clinician may expect. Reports of the histology of Kveim responses should be descriptive of the findings, and their significance interpreted accordingly, well formed epithelioid granulomas being more consistent with a diagnosis of sarcoidosis than granulomatous responses containing few or poorly defined epithelioid cell foci.

These difficulties in interpretation should not be exaggerated, since concordance in reading when criteria of "positive", "negative" and "equivocal" responses are predefined are reported to be 75–87%,^{18,19} and many separate studies of Kveim responses in different groups of subjects have produced patterns of reactions interpreted as positive which are highly consistent, not only within populations but internationally.

PATTERNS OF KVEIM RESPONSIVENESS IN SARCOIDOSIS AND OTHER GROUPS

It is a frustrating fact that the greatest likelihood of a positive Kveim response, with a significant papule and well defined epithelioid granulomas, is found in circumstances when the clinical diagnosis of sarcoidosis is easiest. Thus in Löfgren's syndrome, where the concurrence of fever, erythema nodosum, uveitis, arthralgia, and bilateral hilar lymphadenopathy

allows a confident clinical diagnosis of sarcoidosis, the Kveim test is positive in 70–90% of cases.²⁰ When pulmonary sarcoidosis presents with hilar lymphadenopathy, but either without symptoms or with radiological evidence of lung infiltrates, the subjects are almost as frequently Kveim positive. However, interstitial lung disease due to sarcoidosis without overt lymphadenopathy (radiological category 3) is less easy to diagnose clinically, and in these circumstances only 34–44% of cases yield an unequivocally positive Kveim test result.^{20,21} In clinically lone extrathoracic sarcoidosis, such as disease with manifestations concentrated in the skin, central nervous system, eyes, salivary glands, or abdominal viscera, overall positive response rates have varied from 26% to 50% in the largest studies. Moreover, the frequency of positive responses wanes with increasing chronicity, irrespective of disease activity. Of subjects with sarcoidosis of less than 2 years' known duration, in all 62% are Kveim positive; in disease of more than 2 years' duration the figure falls to 38%.^{10,22} A small proportion of individual cases remain positive for long periods (7% after 13 years) but this persistence of response has no prognostic significance.¹⁷

Positive Kveim responses to adequately validated suspensions can be found in circumstances other than sarcoidosis. It is inappropriate to call these reactions "false positives" when the mechanism of the response is unexplained, and their existence needs to be recognised when interpreting Kveim test results. Amongst healthy controls, various studies have shown an incidence of unequivocally positive responses of 0.7–2%. Of more practical importance are positive responses occurring in disease groups, especially when the presentation may overlap with that of sarcoidosis.

In respiratory practice, the most important distinctions to be made are with tuberculous or lymphomatous lymphadenopathy. Pulmonary tuberculosis is usually Kveim negative, but occasional positive responses in microbiologically proved disease have been reported. These may be more common when indolent pulmonary inflammation and lymphadenopathy resembles sarcoidosis.^{23,24} Such findings are not surprising in that many cases of sarcoidosis and tuberculosis appearing in sequence (and in either order) have been reported.¹² Thus an occasional case with sarcoid like clinical features from which *Mycobacterium tuberculosis* is isolated may yield a positive Kveim test, which in these circumstances is not decisive in diagnostic categorisation. Israel and Goldstein²⁵ also reported positive responses in tuberculous lymphadenopathy, but more disturbingly found positive responses in two cases of chronic lymphatic leukaemia with lymphadenopathy. However, the adequacy of the test suspension which they used was subsequently questioned,^{26–28} and there is no

other evidence that leukaemic or lymphomatous lymphadenopathy is associated with positive Kveim tests. In beryllium disease, another lung disorder with features overlapping sarcoidosis, there is no report of positive Kveim responses, but the only systematic studies²⁹⁻³¹ were carried out in chronic disease. Kveim tests might equally be negative in sarcoidosis of comparable duration.

Overlap of presentation is less of a problem in extrathoracic disease. Positive tests have been reported in chronic brucellosis,³² but this can be distinguished serologically from sarcoidosis. Carefully validated Kveim suspensions have also yielded granulomatous responses in about 50% of cases of recent, active and untreated Crohn's disease of the colon. Seven per cent of patients with ulcerative colitis and half of a smaller group (10) with coeliac disease also responded.³³ In a contrary report,³⁴ Siltzbach found no positive responses in Crohn's disease. This discrepancy is unresolved, but it is probable that the selective production of reactions in some granulomatous and non-granulomatous disorders other than sarcoidosis is a property of some suspensions only. In practice it is rarely difficult to distinguish these disorders from sarcoidosis.

THE KVEIM RESPONSE AND OTHER TESTS IN THE DIAGNOSIS OF SARCOIDOSIS

The diagnosis of sarcoidosis is a statement of belief or knowledge that non-caseating granulomas or their hyalinised remnants are present in a number of affected organs or tissues.¹² The diagnostic "acid test" is adequate biopsy of one or more sites of involvement, and at present evidence other than that of histology is of corroborative value only. Some considerations in previous sections may give the impression that, in clinical use, the Kveim test has more drawbacks than advantages, and in the diagnosis and evaluation of possible sarcoidosis should give way to newer tests whose results are more rapidly available.

These tests include measurement of serum angiotensin converting enzyme (SACE), and in pulmonary disease gallium 67 scanning, bronchoalveolar lavage and transbronchial biopsy. It is well established that subjects with active disease of short duration, those in whom Kveim responses are most often positive, tend to have markedly elevated SACE levels, high ⁶⁷Ga uptake, bronchoalveolar pleocytosis and lymphocytosis,^{35,36} and in this group transbronchial biopsy gives a high diagnostic yield of granulomas.³⁷ In studies of probable sarcoidosis in which direct comparison of Kveim testing and SACE measurement^{35,38} or transbronchial biopsy³⁷ have been made, the predictable high correlation has been confirmed. However, in all studies there were cases in which one test was negative but the other, being posi-

tive, provided support for a diagnosis of sarcoidosis. The Kveim test therefore assesses a parameter distinct from the presence of granulomatous lesions as reflected by SACE or transbronchial biopsy, and is complementary to both.

Biopsy of an involved organ has been facilitated in the case of the lung but remains harder to perform in other circumstances, such as in unexplained cardiac disease, for example cardiomyopathy or conduction defects. Possible sarcoidosis whose manifestations are purely or primarily neurological is another problem: even in clinically compatible circumstances, pleocytosis of the cerebrospinal fluid and lesions demonstrable by computed tomography or nuclear magnetic resonance scanning fall well short of diagnostic confirmation, and a Kveim test when positive is valuable. When granulomas are found unexpectedly or as isolated abnormalities in hepatic or renal disease, a positive response bolsters a tentative diagnosis of sarcoidosis. Even when tissues are readily accessible to primary biopsy, Kveim testing can still help: lymphomas can produce a granulomatous response in adjacent lymph nodes which is indistinguishable from sarcoidosis. Likewise, a small transbronchial biopsy can make a firm diagnosis of granulomatous disease without being adequate to exclude tuberculosis or other cause. In both cases, a positive Kveim test moves the balance of probabilities firmly towards a diagnosis of sarcoidosis.

The diagnostic value of a positive Kveim response is therefore to put on a firmer foundation the "statement of belief" that a constellation of symptoms, signs and compatible findings is due to sarcoidosis. It is a relatively non-invasive test with a value, when well formed epithelioid granulomas result, equivalent to the finding of such granulomas on biopsy of a single organ or tissue remote from those principally affected.¹² A negative Kveim test cannot exclude the diagnosis, but may make it less likely. This can be important, for example in unexplained unilateral hilar lymphadenopathy. It should be borne in mind that corticosteroid treatment will suppress all but the most vigorous positive Kveim responses, and where possible treatment should be delayed. Finally, an equivocal or even clearly granulomatous positive Kveim test is an occasional finding when sarcoidosis is unlikely on other grounds, and in these circumstances it need not cause undue diagnostic anxiety.

Scientific use and application in the study of sarcoidosis

RELATIONSHIP OF THE KVEIM RESPONSE TO SARCOIDOSIS

Kveim induced granulomas in positive responses resemble their spontaneous counterparts in almost

all aspects of light and electron microscopic structure.³⁹⁻⁴¹ Such differences as there are, for example a lack of intracellular inclusions, are attributable to the relative youth of the Kveim induced lesions, since these appear in responses left for a year or more.⁴¹ Cellular composition and phenotype, as characterised by monoclonal antibodies,⁴²⁻⁴⁴ are also closely comparable to the spontaneous lesions of the disease, especially those in the skin.

Two principal hypotheses have been advanced to explain why intradermal injection of a suspension of sarcoid tissue causes a sarcoid like response in subjects with the disorder. The more appealing suggestion, advanced by Kveim,² is that there is in active suspensions an antigen, perhaps derived from an aetiological agent, to which the sufferer has been sensitised in acquiring the disorder. Several lines of evidence support this idea. The gradual formation of granulomas in a positive Kveim response has a similar time course to the Mitsuda reaction to the lepromin test.⁴⁵ This reaction, in which epithelioid granulomas also form, is typical of tuberculous leprosy in which immunity to *M leprae* is strong, as opposed to lepromatous leprosy, in which it is weak.⁴⁶ There is also an apparent kinship between the Kveim response and granulomatous reactions to beryllium and zirconium in individuals specifically sensitive to these metals.⁴⁷ Cellular hypersensitivity is implicated in epithelioid granuloma formation in animal models, and indeed these organised granulomas are sometimes called hypersensitivity type granulomas to distinguish them from the non-immunological, disorganised "foreign body" type.⁴⁸⁻⁴⁹

It is therefore reasonable to suggest that the Kveim response is the result of a cellular hypersensitivity to a component of the suspensions, the nature of which remains elusive. Kveim suspensions are undoubtedly antigenic, at least for rabbits^{50,51} and mice,⁵² and may contain antigens not present in normal spleen tissue. This does not, however, mean that such antigens are of non-human origin, since they may be expressed only in diseased tissue, or that they have a role in the reaction. The term "Kveim antigen" is best avoided until such an entity is shown to be relevant to the Kveim response in man.

An alternative hypothesis, championed by Kooij,⁵³ is that a positive Kveim response is a manifestation of an innate or acquired predisposition to granuloma formation in response to antigenic challenge which is also responsible for granuloma formation elsewhere (the so-called "*terrain sarcoidique*"). Supporters of this notion cite the epithelioid granulomas which sometimes infiltrate old surgical scars in sarcoidosis. A variant of this hypothesis is that a granulomatous response may be due to extensive granulomatous dis-

ease elsewhere. In a murine model, epithelioid granuloma derived lymphokines administered intraperitoneally can convert pulmonary granulomas forming around inert beads from the foreign body type to the epithelioid "hypersensitivity" type.⁵⁴ In its simplest form, this hypothesis would imply that any disease with extensive epithelioid granulomas should cause a positive Kveim response, whereas sarcoidosis with a light granuloma load would not. These deductions appear at odds with clinical experience.

The two main hypotheses are by no means mutually exclusive: sarcoidosis may be an idiosyncratic hypersensitivity response in a susceptible individual to an agent which in others results in absent, clinically inapparent, or even unrelated manifestations of disease. The Kveim response may thus depend on both antigens derived from such an agent *and* an atypical host response. Whatever hypothesis is preferred, the mechanism of the response is of direct relevance to the pathogenesis of sarcoidosis.

BIOLOGICAL ASPECTS OF ACTIVE KVEIM SUSPENSIONS

Materials tested for activity have been derived almost exclusively from tissues affected by the disorder, since early experiments suggested a high yield of effective reagents. However, as noted above, not all lymph nodes and spleens from sarcoidosis are satisfactory: of spleens critically assessed before diagnostic use, over half are rejected because of either low potency or poor selectivity or both. Efficacy cannot be predicted from the histology of the tissue, since both spleens with exuberant granuloma formation and extensive hyalinisation have provided potent suspensions.¹² Furthermore, some tissues provoke an acceptably large number of non-specific inflammatory or even granulomatous responses in control subjects. Conversely, while most normal human tissues produce inactive suspensions,^{3,5,6,55} Kenney and Stone,⁵⁶ in a careful controlled study, found that five of 14 sarcoid subjects, but none of 10 controls, produced relatively feeble but nonetheless definite positive responses to suspensions made from normal human lymph nodes. Together the findings suggest that the response depends on a selective sensitivity to components present in sarcoid tissue, perhaps also found to a lesser extent in normal tissue. They might alternatively indicate simply a generalised predisposition to granuloma formation to intradermal challenge of any form, but a miscellany of organic and inorganic materials used for testing have been found to be ineffective.⁶ A possible exception is a report that typhoid/paratyphoid vaccine produced positive responses in all of six subjects with Löfgren's syndrome.⁵⁷

Two other observations are of interest. Firstly, the

“active principle” can be biologically concentrated. Putkonen⁵⁸ made active Kveim suspensions from sarcoid lymph nodes both before and after *in vivo* corticosteroid treatment. Steroids suppressed the granulomas, but suspensions made from the post treatment nodes were weight for weight nine times more potent than those from pretreatment nodes. Secondly, Kveim responses can be self perpetuating. Siltzbach⁵⁹ used positive Kveim responses as sources of Kveim suspensions, reinjecting them into the same subject and repeating the procedure with each successive papule. Responses of significant magnitude persisted through three cycles, more than could be accounted for by the calculated residue of the original potent suspension. One of us (DN Mitchell, unpublished data) has made similar observations. These findings give rise to intriguing speculation, since Putkonen’s report implies that granulomas themselves are not responsible. It is unlikely that persistent reactions were due to an increasing hypersensitivity counteracting the dilution of a putative antigen, as repeated testing has not been shown to increase the size of Kveim responses. Perhaps a tendency to granuloma formation in response to intradermal challenge is more important than absolute antigenic content, but there are the possibilities that the amount of “antigen” present in Kveim test sites is increased in positive responses, either by replication or by enzymatic modification of a more prevalent precursor. Finally, in subjects with active sarcoidosis, antigenicity necessary for granuloma formation might be provided not from or not only from the suspension but also from the circulation, amongst serological factors or within cells accumulating at the site of the test.

PHYSICO-CHEMICAL CHARACTERISATION OF KVEIM ACTIVITY

Attempts to isolate an active principle have employed a variety of physical and chemical methods. Granulomagenic activity is insoluble in water and is removed by ultrafiltration.^{3 55} Extraction of lipids,⁵⁵ nucleoprotein precipitation with 2 ml saline,⁸ or exposure to enzymes (pepsin, endonuclease, hyaluronidase or neuraminidase)⁶⁰ do not impair potency. Activity also resists short exposure to boiling water and low pH, but can be destroyed by even brief addition of 2N sodium hydroxide,⁶⁰ autoclaving or prolonged boiling, or vigorous exposure to hot organic solvents.⁶¹ Fractionation of crude spleen suspensions by centrifugation at 2500 g leaves activity in the supernatant, but at 30000 g removes it to the pellet, implying a microparticulate component. Chase and Siltzbach⁶² found optimal activity in the fraction sedimented by centrifugation at 5500 g for 15 minutes, with smaller particles (10000 g) showing less activity. In density gradient centrifugation, activity is

found in fractions equilibrating between 50% and 70% sucrose,⁶³ which on electron microscopy include membrane bound dense bodies, whose associated acid phosphatase activity suggests a lysosomal origin.

Animal models of granulomatous disease indicate that for epithelioid granuloma formation the stimulus must be both antigenic and persistent.^{48 49} Ripe⁶⁴ tested the hypothesis that the particulate nature of active Kveim suspensions was necessary only as a carrier for an otherwise soluble component. He conjugated the cytoplasmic fraction of a centrifuged sarcoid node homogenate to Sephadex beads, and used the conjugate for Kveim testing. Five of 13 patients gave positive responses. None reacted to the cytoplasmic fraction alone; two of 13 subjects produced responses to the unconjugated beads. The approach used was designed to conjugate proteins; other components of the cytoplasm might yield conjugates of greater potency and selectivity.

IMMUNOLOGICAL STUDIES IN VITRO AND IN VIVO

Ability to demonstrate specific sensitivity to components of Kveim material *in vitro* in sarcoidosis would be an important advance for both diagnostic and scientific purposes. Various studies of cellular immunity have employed blast transformation,⁶⁵ DNA synthesis^{66 67} direct⁶⁷⁻⁷⁵ or indirect^{74 76} inhibition of leucocyte migration, or other more complex tests⁷⁷ to try to identify a response. In some, sensitivity in sarcoidosis has been claimed.^{67-71 75-77} Unfortunately, when present, such sensitivity does not correlate directly with *in vivo* Kveim response, and the cells may react only to certain Kveim suspensions.⁶⁷ With indirect migration inhibition, leucocytes from cases of Crohn’s and coeliac disease may also give positive results.^{76 78-80} Methodological variations, including the amount of suspension used, confound direct comparison between these studies.¹² No method has the simplicity necessary for routine diagnostic use, and it is noteworthy that even for scientific application none has triumphed as a standardised “*in vitro* Kveim test.”

Reports of serological reactivity to Kveim suspension are fewer; precipitating⁸¹ and haemagglutinating⁸² antibodies to Kveim material, which are not absorbed out by normal spleen extract, have been identified in sarcoidosis, but they can also be found in control sera. The higher titres in sarcoidosis may reflect the general stimulation of antibody responses which is a feature of the disorder.

Passive transfer of Kveim responsiveness *in vivo* has been attempted. Rogers and Haserick⁵⁵ found that serum from a Kveim responsive patient, admixed with Kveim suspension then used to test a single normal subject, produced a positive response. This

finding has not been confirmed. Webb and Mitchell (unpublished data) stored serum from Kveim positive patients presenting with hilar lymphadenopathy alone. When Kveim responsiveness subsequently waned, it could not be restored by admixture of the thawed serum to the same Kveim suspension. On the other hand, Lebacqz⁸³ identified three (of 22) subjects with sarcoidosis whose leucocytes, injected around Kveim test sites in nine control subjects, were capable of mediating a positive response in a total of seven cases. The potential of this study is unexplored, and ethical considerations make the experiment difficult to repeat.

SEQUENTIAL STUDIES OF GRANULOMA FORMATION

In view of the resemblance of Kveim induced granulomas to spontaneous lesions of sarcoidosis, it is fair to conclude that the routes by which the test induces granulomas reflect the pathogenic processes of sarcoidosis, and that the test can be used as a model of epithelioid granuloma formation in man. The sequence of appearances in developing reactions at light^{12 59} and electron⁸⁴ microscopic levels have been reported, and more recently immunochemical techniques have been used to identify serological factors⁸⁵ and cells^{86 87} in the emerging granulomas. But few studies^{55 88 89} have examined control responses, which is necessary in order to address the important questions of how the cutaneous response to Kveim material in sarcoidosis differs from normal, and how early such differences can be detected.

After the injection of Kveim material there may be macroscopic evidence of mild inflammation in the first few days. Some authors claim that a positive response can be predicted from an early erythematous reaction,^{45 90} but our experience is that positive responses may be initially unremarkable. Microscopically, there is a dermal deposit of amorphous material whose staining characteristics (PAS positive) imply that it is part of the injected suspension.⁵⁹ This is surrounded by local macrophages showing increased lysosomal hydrolase activity.⁸⁸ Following a macroscopically "silent" interval, at about ten days, an erythematous papule begins to appear in those responses destined to produce well formed epithelioid granulomas. The corresponding microscopic appearances include an influx of atypical mononuclear cells,⁵⁹ phenotypically members of the mononuclear phagocyte series.⁸⁹ There is endocapillary deposition of immunoglobulin and complement.⁸⁵ In the centre of the response, macrophages show increasing evidence of activation,^{87 89} including giant cell formation and intense expression of the HLA-DR antigens which are necessary for antigen presentation.⁸⁹ Moreover, lymphocytes begin to accumulate in these

areas, and their immunological phenotype (T4+/Leu8-)^{86 89} is that associated with so called "true helper" T cells.⁹¹ The findings correspond to those in putative "early" lesions of sarcoidosis obtained by transbronchial biopsy.⁹² In emergent Kveim granulomas, electron microscopy shows intimate association of lymphocytes and macrophages.⁸⁴ Suppressor type (T8+) cells tend to remain confined to pericapillary regions, implying that the helper cells are selectively attracted by the macrophage activity. Possibly under the influence of T lymphokines, atypical mononuclear cells, activated macrophages and giant cells undergo phenotypic differentiation,⁸⁹ assuming the characteristics of the mature epithelioid granuloma. Using PAS staining,⁵⁸ or Kveim material labelled with Alcian Blue,⁹³ it is possible to show that at least some of the cells of the resultant granulomas contain Kveim suspension derived material.

Some features of developing positive responses are also found in control responses. These include early macrophage activation, and later endocapillary complement deposition.^{88 89} But while granulomatous accumulations of activated macrophages occur in Kveim positive subjects in response to suspensions of *normal* spleen, in contrast to the response to Kveim material, there is no migration into these areas of T cells, and macrophage maturation into epithelioid cells does not occur.⁸⁹ This implies that a component of Kveim suspension directly or indirectly attracts the T lymphocytes, whose subsequent effects on macrophages cause epithelioid granuloma formation. Nevertheless, other immunological responses to Kveim material appear to be possible. In our own study after about a week some healthy subjects developed papules which contained dense perilesional infiltrates composed mainly of T cells and dendritic cells.⁸⁹ Such infiltrates, whose appearance suggested the expression of an acquired immune response to the implanted suspension, were either absent or retarded in subjects with sarcoidosis. They often resolved by four to six weeks, but probably correspond to the "non-specific" inflammatory responses seen in some mature Kveim reactions, implying that the latter too are immunologically mediated reactions to Kveim suspension, which is of course allogeneic tissue.

ABERRANT CELLULAR IMMUNITY AND THE KVEIM RESPONSE

Among the many unexplained phenomena of sarcoidosis is the common observation that the expression of cutaneous delayed type hypersensitivity is impaired. Since there is good evidence that cellular immunity is active at disease sites,⁹⁴ this paradoxical cutaneous anergy has been attributed to compartmentalisation of immunocompetent cells.⁹⁵ It is, however, clear that

in active cases of pulmonary sarcoidosis the necessary T cells are available to produce cutaneous granulomas in the Kveim response, which occurs in the face of cutaneous "anergy." The paucity in sarcoidosis of the dense perivascular lymphocytic infiltrates seen in some normal subjects in developing Kveim responses⁸⁹ may reflect this coexistence, although there is no direct relationship between failure to respond to recall antigens and a positive Kveim response in sarcoidosis.¹⁰ But, some years ago, D'Arcy Hart and Mitchell⁹⁶ identified a small group of apparently healthy subjects who failed to become tuberculin positive despite twice being effectively vaccinated against BCG; among 12 of these, seven were found to yield positive Kveim responses. This is further evidence that expressions of cellular immunity in the skin as classical delayed type hypersensitivity in tuberculin responses and as epithelioid granuloma formation have distinct mechanisms. Thus traditional *in vitro* tests for cellular hypersensitivity may be inappropriate for use with Kveim suspensions. Moreover, it is not necessary to postulate a compartmentalisation of immune response in sarcoidosis, since the cellular participants in granulomagenesis in disease sites are available at the periphery.

While subjects with sarcoidosis who become anergic to tuberculin often remain so for many years, the frequency of positive Kveim responses inexorably declines, even among subjects whose disease remains active.⁵⁹ In those subjects in whom the response remains positive, the papule produced on retesting is smaller than the original one.⁵⁹⁻⁸⁹ In subjects retested with the same suspension who have become Kveim negative, there is no evidence of immunological activity at the test site.⁸⁹ These observations suggest that if specific sensitivity to Kveim material was present initially it was later suppressed. Spontaneous modulation of granulomatous inflammation,⁹⁷ mediated by suppressor T cells,⁹⁸ is found in animal models of granulomatous disease. In sarcoidosis, a disorder which is in many cases self limiting, we may reasonably propose the existence of similar regulatory mechanisms. More important than their effect in ablating Kveim responsiveness would be the role of such mechanisms in determining whether the disorder pursues a benign or chronic clinical course.

Conclusions

A positive Kveim test is still useful as a simple and relatively non-invasive means of supporting a diagnosis of sarcoidosis, when this is compatible with the clinical presentation and other findings, but the result alone cannot make or exclude the diagnosis. Its mechanism and its relationship to sarcoidosis remain almost entirely mysterious. It is clear that cells of the

immune system play a part in granuloma formation, but not whether the intradermal administration of Kveim suspension provides a unique initiating antigenic stimulus, or merely establishes a theatre in which the remote events of the disease can be re-enacted. Whether or not specific sensitivity to components of Kveim material exists in subjects with sarcoidosis before administration of the test, the effect of intracutaneous implantation of this allogeneic tissue is likely to depend on both the nature of the suspension and the immunological state of the host at the time of testing.

The Kveim response is a central enigma amongst the many peripheral phenomena which accompany sarcoidosis; it will demand explanation until the aetiology of the disorder is laid bare. Elucidation of the response may itself be a means of achieving this objective. Further dissection of soluble and particulate components, to define the minimum requirements for activity *in vivo*, is necessary; the elements of host response, especially those mediating the helper T cell accumulation, which is the earliest indication of a developing epithelioid granuloma, ought to be isolated and characterised. Here, *in vitro* techniques for culturing isolated or cloned immunocompetent cells may be valuable. Finally, sensitive methods of identifying fragments of microbial genome may permit examination of tissue used in the preparation of suspensions for the presence of mycobacteria or other organisms which are candidate aetiological agents for both sarcoidosis and the Kveim response.

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