THE RELATIONSHIP BETWEEN HYPERSENSITIVITY AND IMMUNITY TO VACCINIA*

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The role that hypersensitivity plays in the phenomenon of immunity to infectious agents is controversial. This question has been under investigation since the time that Koch demonstrated that the reinoculation of tubercle bacilli into guinea pigs brought forth responses quite different from the original inoculation, ("Koch's phenomenon") (1). The ulcer which results from the original subcutaneous inoculation heals slowly and is associated with a dissemination of the organisms throughout the body. The lesion produced in the reinoculated animal, which by this time has become sensitive to tuberculin, heals quickly. Moreover, the spread of organisms through the body is limited.

The issue relates to the interpretation of this "phenomenon". Is the tuberculin sensitivity the essential feature of the immunity exhibited by the reinoculated animal? Is it the hypersensitivity which confers this immunity? One may readily point to a number of infectious diseases in which hypersensitivity regularly accompanies some degree of immunity (syphilis, leprosy, animal ringworm, etc.). The question is whether the immunity is dependent on the hypersensitivity or whether these are only associated or parallel phenomena. According to Krause, hypersensitive tissues may provide immunity by their capacity to react violently to the organism, thus confining it to the area of inflammation and preventing its dissemination. (2) Rich believes that the inflammation develops too late to prevent the dissemination of organisms (3, 4). Tubercle bacilli have been shown to spread more rapidly from a site inflamed by the injection of tuberculin than from a normal site (5). Rich has shown that animals rendered highly sensitive to tuberculin by the injection of PPD did not acquire a resistance to the tubercle bacillus. In summary Rich states: "There has never been placed on record one single experiment or clinical observation that demonstrated that hypersensitivity is necessary for the development of resistance in any stage of tuberculosis or any other infection under any condition whatsoever" (4).

No where is the confusion about the role which hypersensitivity plays in the development of immunity better illustrated than by the various interpretations placed on the responses resulting from vaccinia inoculation of animals and of humans for smallpox vaccination. In general the sequence of events in animals reinoculated with vaccinia virus parallels that seen in tuberculosis; that is, on primary inoculation a pustular lesion develops which reaches a peak at about 5 to 6 days and then undergoes involution with healing complete in about two

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Presented at the Twelfth Annual Meeting of the Society for Investigative Dermatology, Inc., Atlantic City, New Jersey, June 7, 1951.

weeks. On revaccination the lesion reaches a peak between 48 and 72 hours. It is more severe than the original inoculation and healing is more rapid, being completed in about six days. Much of the confusion about this subject is due to the failure on the part of many observers to define precisely their criteria of immunity. For example, Bland regards immunity in the guinea pig, rabbit and monkey as "no reaction to the injection of active virus at any dilution" (6). Broom considers that in humans the length of the interval between the inoculation and the development of the peak of reaction is the essential criterion for immunity (7). Still other workers have disregarded the skin reaction entirely and rely on the demonstration of circulating neutralizing antibodies as evidence of the immune state. In the present report we have arbitrarily assumed that immunity is demonstrated by the rapidity of healing of the experimental lesion. This may not hold for other diseases. In vaccinia, however, it may be assumed that healing cannot occur until the virus can no longer proliferate freely and is finally eliminated. The healing time thus crudely measures the forces which prevent multiplication of the virus.

The particular purpose of this study was to determine if sensitization *per se* is an essential component of immunity.

METHODS AND MATERIALS

White guinea pigs weighing about 700 grams were used throughout. Areas to be used for inoculation or skin testing were prepared 48 hours beforehand by plucking the hair. The active virus material was pooled from several passages in the chick embryo and all virus that was used in these experiments was taken from this original pool. The virus was of a potency of about 10^8 infectious units per ml. when titered by inoculation on the chorioallantoic membrane of the chick embryo. The antigen used for skin testing was obtained by exposure of the full strength active virus suspension to ultra-violet radiation for 30 minutes at a distance of 3 inches, with constant agitation during the exposure. This killed the virus as demonstrated by the failure of the treated suspension to produce lesions on the chorioallantoic membrane. The animals were divided into three groups. The first group was the uninfected controls "immunized" in the manner described below (Group I). The second group was the infected controls. These animals were inoculated subcutaneously on both sides with 0.1 ml. of the full strength live vaccinia virus suspension twenty-one days before the main experiment was begun. The infection was allowed to run its course. The third group, or the infected desensitized group was prepared in the same way as the infected controls. In addition, on the twenty-third day after the original inoculation, when healing had been complete for about 8 days, the desensitization program was begun. This involved the daily subcutaneous inoculation of .2 ml. of full strength killed vaccinia virus suspension up to the thirty-eighth day. Thereafter, the amount was increased to 0.5 ml. daily. An exactly similar course of killed virus injections was given to the uninfected controls (Group I). This was done both to see if sensitization could be established by the daily inoculation of a large quantity of killed virus as well as to determine its "immunization" value in protecting animals which had never been infected. The infected control group (Group 2) was not desensitized. The animals in each group were skin tested with 0.1 ml. killed vaccinia antigen given intracutaneously on the 21st, 35th, 42nd, and 49th days after the original inoculation of live virus. On the 51st day after the original inoculation of live vaccinia virus, the previously infected animals were reinoculated with .1 ml. of live vaccinia virus intracutaneously, and the animals not previously infected (Group I) were similarly inoculated for the first time.

TABLE I

Results of skin tests on previously prepared guinea pigs using killed vaccinia antigen
(Desensitization begun on 23rd day)

GROUP	METHOD OF PREPARATION	NUMBER OF ANIMALS	DAYS AFTER INOCULATION			
			21	35	42	49
1	Uninfected, "immunized" controls†	10	0*	0	0	
2	Infected controls	5	1.0	0.8	0.8	0.7
3	Infected and desensitized	9	0.8	0.7	0.2	0.1

* Each figure represents the average diameter of the lesions in that group expressed in centimeters.

† Treated after the 23rd day in the same manner as the infected, desensitized group (group 3).

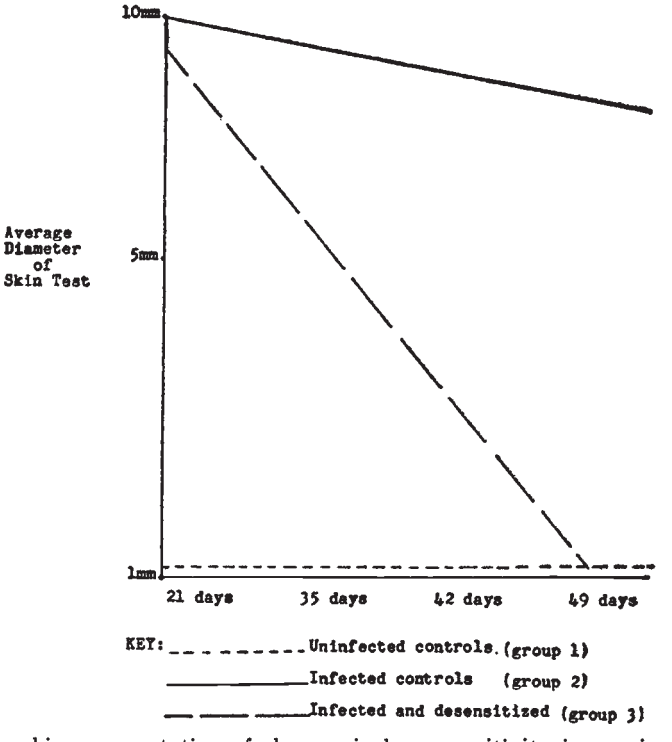

FIG. I. Graphic representation of changes in hypersensitivity in previously prepared guinea pigs.

RESULTS

The results of skin testing during the desensitization program are shown on Table I. It will be noted that no positive reactions were elicited in the control group given a course of killed antigen. In short these animals did not become sensitized (Group I). There was a gradual fall in skin sensitivity after the 21st

day in the Group 2 animals. This was slight and in the direction anticipated with the passage of time. It should be pointed out that there was often a fairly marked variation in the skin test responses among the animals of the same

TABLE II

Results of inoculation of live vaccinia on 51st day after preparation of guinea pigs

GROUP	METHOD OF PREPARATION	NUMBER OF ANIMALS	PEAK OF REACTION	TIME TO HEALING	REACTION 24 HOURS	REACTION 48 HOURS
			hrs.	*days*		
1	Uninfected controls	10	144*	16	0	0.5 cm. papule
2	Infected controls	5	48	8	0.5 cm. papule	0.9 cm. necrotic papule
3	Infected and desensitized	9	72	9	0.5 cm. papule	0.8 cm. necrotic papule

* Each figure represents the average for that group.

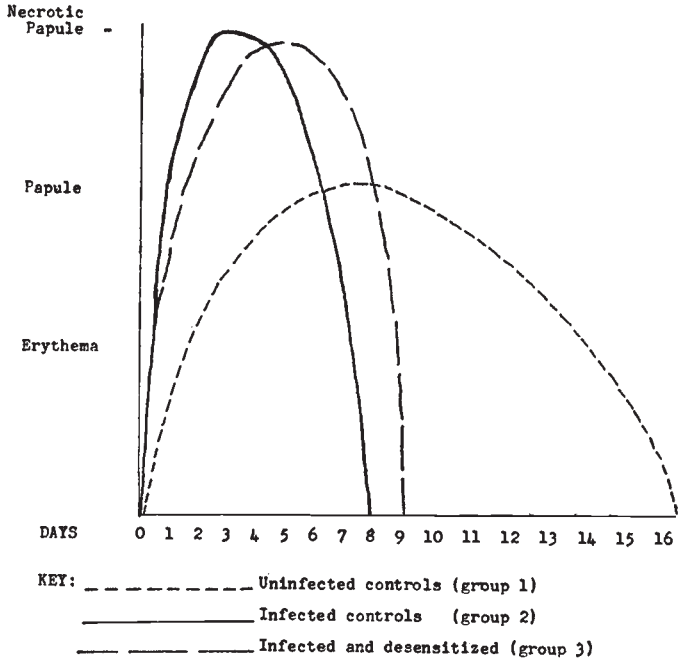

FIG. II. Graphic representation of course of reaction following the injection of active vaccinia virus into previously prepared guinea pigs.

group and even in the same animal on repeated testing. In the infected and desensitized group (Group 3), however, there was a marked loss of hypersensitivity to the infectious agent. This is graphically shown in Figure I. Two of the animals did not become completely desensitized despite the enormous amount of dead antigen injected. We have considered that the results of skin

testing guinea pigs are very erratic and that the measurements given have only a general significance in showing trends. Table II records the result following the inoculation of live vaccinia on the 51st day, following the completion of the desensitization program. In the uninfected "immunized" controls (Group I) the peak of the reaction was reached at 144 hours and the healing time was 16 days. The reaction was similar to that observed in normal untreated animals inoculated for the first time. In short, no immunity was induced by a course of killed virus injections. In the infected but not desensitized controls (Group 2), the peak of the reaction was reached at 48 hours and the healing time was much shortened. This indicated immunity as was expected. The lesion was much more severe than that of the uninfected controls. In the infected and desensitized group (Group 3), the reaction was similar in every way to that in the infected controls, although there was an absence of cutaneous hypersensitivity with dead virus.

DISCUSSION

The results seem to indicate that cutaneous hypersensitivity and immunity may be dissociated experimentally; that is, one can eliminate the hypersensitivity of the skin and yet not interfere with the immune process. These findings with the vaccinia virus are in a general way in support of the thesis of Rich and his coworkers, who have made similar observations with the tubercle bacillus. Tuberculosis and vaccinia, however, are not comparable diseases.

Vaccination of humans against smallpox is a time-honored procedure; yet, the interpretation of the responses of humans to such vaccination is often confused. It is important that the cutaneous reactions (or lack of reaction) obtained by inoculating vaccinia virus into previously vaccinated individuals be soundly evaluated. The findings of the present study bear directly on this problem.

The issues to be resolved are: (1) What is the meaning of a "no take" in a human known to have been successfully vaccinated in the past. (An unofficial survey in our own hospital elicited the response from many physicians that "no take" indicated a solid immunity); (2) If a reaction does occur, does this mean that the level of immunity will be "boosted". In other words, does a reaction increase the degree of immunity?

Returning to the first question, one may note that the experimental data furnishes a decisive answer. Some degree of inflammatory cutaneous reaction *always* occurred when vaccinia virus, *live or dead*, was injected into previously infected animals. It is evident that the complete lack of a reaction ("no take") could occur only if the virus failed to be injected below the stratum corneum. Previously infected animals acquire a sensitivity to vaccinia antigen in exactly the same manner as tuberculous animals develop a sensitivity to the tubercle bacillus. Therefore, the injection of even dead virus into a previously vaccinated animal will give a reaction which may be interpreted as a positive skin test. Such a reaction is simply an indication of previous infection. In previously vaccinated humans, the "no take" reaction is certainly not an indication of immunity but is probably an indication of improper technic; that is, the virus

was not actually inoculated below the stratum corneum. Thus, the "no take" reaction has no significance whatsoever in terms of indicating immunity. When a "no take" is obtained, the vaccination obviously must be done over.

On the other hand, coming back to the second question, how are positive reactions in previously vaccinated humans to be interpreted? It is at once clear that the mere elicitation of a positive reaction does not insure that the level of immunity will be boosted. If the virus is dead, for instance, the reaction will simply be a positive skin test which probably does not significantly raise the level of immunity although it does indicate that the individual has previously been infected. Presumably live virus must be inoculated to raise the existing level of immunity. The question then arises as to how one differentiates a positive skin test reaction (dead virus) from a truly immunizing reaction (live virus). The experimental data furnishes a reasonably satisfactory answer. The course of the cutaneous response to the injection of live virus differs from that following the injection of dead virus. Initially, within the first 48–72 hours the reactions are roughly the same. Thereafter, the skin test reaction with dead virus wanes, whereas the true immunizing reaction continues to become more inflammatory. The immunizing reaction has a later peak which, incidentally, tends to be more severe. The skin response to the immunizing action of live virus requires at least twice as long to return to normal as the positive skin test to dead virus. The distinction thus essentially rests on a time sequence.

The same conclusions have emerged from Beneson's work on human beings (8). Hooker's earlier work in 1929 can now be more accurately reinterpreted (9). This latter worker showed that there was a negative skin test reaction to killed virus in those who would develop a primary take (non-immune reaction) following the inoculation of living virus. Correlatively, a positive skin test could be elicted in those who would develop the immune reaction following inoculation of living virus. Hooker believed that a positive skin test was thus a sign of immunity. One must point out, however, that a positive skin reaction is a test of hypersensitivity and not of immunity per se. It is true that hypersensitivity is often associated with immunity and is, at any rate, a sign of past infection. The prior infection undoubtedly establishes some degree of lasting immunity and it is only in the sense that a positive skin test indicates past infection that one can regard it as a reflection of the immune state. Regan has recently described in humans an immediate white papule which appeared in 3–10 minutes following a successful inoculation (10). We were unable to observe this type of reaction in guinea pigs.

CONCLUSIONS

1. The desensitization of guinea pigs previously infected with vaccinia virus did not result in a loss of immunity.

2. Previously infected animals always exhibited a cutaneous response whether inoculated with living or dead virus.

3. The responses of humans to reinoculation with vaccinia virus are discussed with relation to their influence on the immune state.

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DISCUSSION

Dr. Baldridge, *Philadelphia:* If one looks upon allergy as any specific alteration in the capacity to react, then immunity becomes a special case of allergy and the distinction between the two phenomena becomes less important. Therefore, I agree that the term "specific hypersensitivity" in a presentation of this sort is preferable to the use of the designation "allergy".