CELL-MEDIATED IMMUNITY AND ANTIBODY RESPONSES IN THE RESPIRATORY TRACT AFTER LOCAL AND SYSTEMIC IMMUNIZATION*

By ROBERT H. WALDMAN, M.D., and CHRISTOPHER S. HENNEY, Ph.D.

(From the Department of Medicine, University of Florida, Gainesville, Florida 32601, and the Department of Medicine, The Johns Hopkins University, Baltimore, Maryland 21205)

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Humoral immune responses in external secretions after local antigenic stimulation have been widely reported (1, 2) and the important protective role of such responses in maintaining mucosal surfaces free from infection well established (3, 4). Humoral responses in external secretions are characterized by the predominance of antibody of the IgA class, distinguishable from the majority of serum IgA by the additional possession of a polypeptide known as secretory component (5).

It has only recently proven possible to demonstrate cell-mediated immune responses in external secretions, and this in the respiratory tract of guinea pigs after nose drop administration of aqueous dinitrophenylated human IgG (6).

The present study was undertaken to compare the nature, extent, and specificity of both cell-mediated and humoral immune responses in the upper respiratory tract of guinea pigs after both local and systemic administration of antigen, and to contrast such responses with those observed in the serum of the same animals.

Additionally, in order to investigate the interrelationships that exist between systemic and local immune responses, the ability of tolerant guinea pigs to mount immune responses in their external secretions to the tolerizing antigen was studied.

Materials and Methods

Antigens.—Human γG globulin (HGG)[1] was prepared from pooled normal serum by diethylaminoethyl (DEAE)-cellulose (Mann Research Laboratories, New York) chromatography. The fraction eluted with 0.005 M phosphate buffer, pH 8.0, was collected and concentrated by ultrafiltration. The preparation was shown to be homogeneous by gel diffusion precipitin analysis and by immunoelectrophoresis, employing a goat antiserum against whole human serum.

* Much of this work was carried out at the World Health Organization Immunology Research and Training Center, Lausanne, Switzerland.

[1] *Abbreviations used in this paper:* DNP-HGG, dinitrophenylated HGG; HGG, human gamma G globulin; MIF, macrophage-inhibition factor.

The HGG preparation was dinitrophenylated by the method of Eisen et al. (7). To a solution containing 100 mg of protein in 5 ml of phosphate-buffered saline was added 100-fold molar excess of 2,4-dinitrobenzene sulfonic acid dissolved in 1 ml of 1 M Na_2CO_3. The pH of the reaction mixture was adjusted to 10.4 by the further addition of 1 M Na_2CO_3, and the mixture was then rotated gently for 24 hr at room temperature. The number of dinitrophenyl (DNP) groups per molecule of protein was calculated on the basis of DNP having a molar extinction coefficient at 360 nm of 17,400, and HGG an assumed molecular weight of 160,000. The sample used throughout this study had 11.2 DNP groups/molecule of protein.

Animals.—Guinea pigs of the Hartley strain, weighing approximately 400 g, were used.

Antisera.—Guinea pig $\gamma 2$ globulin was isolated as that fraction of normal guinea pig serum eluted from DEAE-cellulose using 0.01 M phosphate buffer, pH 8 (8). Digestion of this material with papain gave characteristic Fab and Fc fragments. The Fc fragment was isolated by Pevikon block electrophoresis and used directly to immunize rabbits. The resulting antisera were specific for guinea pig $\gamma 2$ globulin.

Rabbit antisera specific for guinea pig $\gamma 1$ globulin were prepared as previously described (9). Guinea pigs were immunized with ovalbumin in complete Freund's adjuvant and the $\gamma 1$ globulin fraction of the antisera was obtained by block electrophoresis. Rabbits were immunized with this $\gamma 1$ globulin fraction included in complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.). The resulting rabbit antiserum gave both $\gamma 1$ and $\gamma 2$ precipitin bands with the guinea pig antiserum on immunoelectrophoresis.

An antiserum reacting with $\gamma 1$ globulin but not with $\gamma 2$ globulin was prepared by absorbing the antiserum with the $\gamma 2$ globulin preparation from normal guinea pig serum.

An antiserum specific for guinea pig IgA was kindly provided by Dr. R. Asofsky, National Institute of Allergy and Infectious Diseases, Bethesda, Md. The serum was raised in goats by immunization with an IgA protein preparation isolated from colostrum.

Labeling of Proteins with ^{131}I.—Protein preparations were labeled with ^{131}I by the chloramine-T method of McConahey and Dixon (10).

Measurements of Antigen-Binding Capacities.—The antigen-binding capacities of whole serum samples and bronchial washings, and the relative contribution of $\gamma 1$, $\gamma 2$, and γA globulins to this total, were estimated as previously described (11). Radioactive antigen was added to the serum together with an excess of nonradioactive antigen. After incubation at 37°C for 4 hr, an excess of the appropriate rabbit antiserum was added, sufficient to cause total precipitation of the immunoglobulin fraction under study. The precipitates were allowed to stand overnight at 4°C and were then washed twice with cold barbital-buffered saline, pH 8, and then counted in a well-type crystal machine model C 120-1 (Nuclear Chicago Corp., Des Plaines, Ill.). The final counts per minute recorded were corrected for normal serum controls and tabulated as the maximal amount of antigen (in micrograms) that immunoglobulin bound in 1 ml of serum. This calculation followed from knowledge of the amount of antigen present in the mixture and the percentage of this amount which was immunoglobulin bound in extreme antigen excess.

Immunoglobulin Determinations.—$\gamma 1$, $\gamma 2$, and γA globulin levels in serum and bronchial washings were determined by a radial diffusion immunoprecipitation technique (12) employing isolated $\gamma 1$ and $\gamma 2$ immunoglobulin standards. $\gamma 1$ immunoglobulin was a gift from Dr. John Cebra, The Johns Hopkins University; the $\gamma 2$ protein preparation was isolated in the manner described. γA globulin levels were calculated on an arbitrary unitage using a pooled sample of unimmunized guinea pig serum as standard.

Immunization Protocol.—Groups of 12 animals were each immunized by one of the following schedules: (*a*) immunization via the rear footpads with 40 μg of dinitrophenylated human IgG (DNP-HGG) in complete Freund's adjuvant; (*b*) lightly anesthetized (0.15 ml of 1:10 Nembutal, intraperitoneally) animals were given approximately 200 μg of the same antigen

in 0.25 ml phosphate-buffered saline, pH 7.4, by nose drops; (*c*) intravenous administration of 5 mg of DNP-HGG just before immunization by nose drops as in (*b*) above.

Preparation of Cell Populations.—Three animals from each immunization group were exsanguinated 7, 15, 19, and 26 days after antigen administration and the spleens and the tracheae, bronchi, and lungs were removed. The lower respiratory tracts were externally washed and 5 ml of sterile Eagle's medium was instilled into the main stem bronchi in one ml portions. The cell suspension aspirated from the lungs was centrifuged at 1500 rpm for 5 min. The supernatant was removed and immediately frozen. The cell pellet was resuspended and washed three times with Eagle's medium containing 10% inactivated calf serum. The final suspension was found to contain between 3.1 and 4.0 $\times$ 10^6 lymphocytes, and between 4.6 and 7.5 $\times$ 10^6 macrophages per animal. The supernatant fluid was dialyzed against distilled water, lyophilized, and resuspended in 1 ml of phosphate-buffered saline, pH 7.4.

Spleens were homogenized by hand in a glass grinder containing modified Eagle's medium containing 4-fold amounts of amino acids and vitamins. The cell suspension was centrifuged at 1500 rpm for 5 min and the sediment was resuspended and allowed to stand for 1 hr at 4°C. The cells remaining in the supernatant were sedimented by centrifugation at 1500 rpm for 5 min and resuspended in 0.83% sterile NH_4Cl for 30 min to induce erythrocyte lysis. The remaining cells were again sedimented and washed twice in Eagle's medium containing 10% inactivated calf serum. The number of viable lymphocytes was determined by hemocytometer counting using the trypan blue exclusion test.

Macrophage Inhibition Test.—Lymphocyte preparations were assessed for the presence of specifically sensitized lymphocytes by estimation of their capacity to inhibit, in the presence of DNP-HGG, the migration of "normal" guinea pig macrophages. The method used was that previously described in detail (13). Normal guinea pig peritoneal exudate cells were harvested aseptically 48 hr after the administration of 20 ml of paraffin oil intraperitoneally. The average number of macrophages thus obtained was 1.3 $\times$ 10^8 per animal. Splenic lymphocytes were mixed with peritoneal macrophages either in Eagle's medium containing 10% inactivated calf serum or in the same medium containing 300 μg/ml of DNP-HGG, so that the final suspension contained 25 $\times$ 10^6 macrophages/ml and 5 $\times$ 10^6 lymphocytes/ml. Capillary tubes were filled from each cell suspension and sealed with paraffin wax. The filled tubes were centrifuged at 900 rpm for 2 min and then cut cleanly at the cell interface and mounted into wax paraffin chambers filled either with Eagle's medium containing 10% inactivated calf serum or the same medium containing 300 μg/ml of DNP-HGG. The chambers were then stored in a CO_2 incubator at 37°C for a total of 48 hr. After this time the extent of migration was assessed by projecting the migration areas onto a glass screen and tracing these areas onto fine grade paper. The traced areas were then cut out and weighed. The effect of antigen on migration was assessed by calculating the migration in the presence of antigen as a percentage of that obtained in its absence.

Similar studies were made with lymphocytes obtained from the bronchial washings of animals from each group. Individual washings were adjusted to contain 5 $\times$ 10^6 lymphocytes and 25 $\times$ 10^6 macrophages/ml by the addition of normal guinea pig peritoneal exudate cells. The effect of the antigen on the migration of these suspensions of macrophages and lymphocytes was assessed in a manner identical to that used for the spleens.

RESULTS

Humoral Immune Response.—Immunoglobulin levels in serum and bronchial washings were measured by single radial diffusion analysis in agarose gels (12). γ1 and γ2 globulin levels were measured using the isolated immunoglobulins as standards. γA globulin was measured relative to a pooled normal guinea pig

serum standard to which was attributed an arbitrary value of 100 γA immunoglobulin units.

Antibody to DNP-HGG in the serum and the cell-free fluid obtained from bronchial washings from the three groups of animals was measured by radioactive antigen binding (Tables I and II). In each case the amount of DNP-HGG–^{131}I specifically bound to γ1, γ2, and γA globulins was individually assessed. 10 μg of antigen was mixed with 0.1 ml 1:10 serum (or the same volume of undiluted bronchial washing) and after incubation at 37°C for 4 hr, 0.2 ml

TABLE I

Immunoglobulin Levels and Specific Antigen-Binding Characteristics of Immunoglobulin G Fraction of Serum and Bronchial Washings

Guinea pigs‡	Immunoglobulin levels				Specific antigen-binding levels			
					μg of antigen* bound/mg of			
	γ1		γ2		γ1		γ2	
	Bronch. wash.	Serum	Bronch. wash.	Serum	Bronch. wash.	Serum	Bronch. wash.	Serum
	(μg)/ml	(mg/ml)	(μg/ml)	(mg/ml)				
Normal	41	2.6	58	3.6	<5	<5	<5	<5
Footpad immunized§	54	5.2	50	4.8	24	104	20	92
Nose drop immunized‖	47	2.8	56	3.6	22	15	20	11
Tolerant animals,¶ Nose drop immunized	48	2.6	60	3.6	10	<5	8	<5

* Micrograms-DNP-HGG–^{131}I specifically bound.

‡ All values represent means of nine estimations on individual samples taken between 7 and 19 days after antigen administration.

§ With 40 μg DNP-HGG in complete Freund's adjuvant via rear footpads.

‖ With approximately 200 μg DNP-HGG in phosphate-buffered saline.

¶ Animals received 5 mg DNP-HGG intravenously on same day as sensitizing dose of antigen.

(containing approximately 250 μg antibody) of specific anti-immunoglobulin antiserum was added. After incubation at 4°C overnight the immune precipitate was removed by centrifugation and, after careful washing, the ^{131}I content was measured. To the supernatant was added anti-immunoglobulin antiserum of another specificity, the precipitate thus formed was again washed and counted, and to the resulting supernatant the third anti-immunoglobulin antiserum was added. The order of addition of antisera was usually anti-γ2, anti-γ1, anti-γA but reversing the order was found not to affect appreciably the amount of ^{131}I bound to each fraction. In each case the micrograms of antigen bound per milligram of immunoglobulin or per unit of γA globulin was calculated. The relative contributions of each antibody class to the total specific antigen binding was also calculated by expressing the micrograms of antigen

bound to a particular class per milliliter of serum (or bronchial fluid) as a percentage of the total amount of antigen that immunoglobulin bound. The greatest antibody response was demonstrable in the serum from the animals which received the antigen subcutaneously (Table I). There was very little antibody demonstrated in the sera of the animals which were immunized by nose drops ($P < 0.01$) as compared with the subcutaneous group. No antibody was detectable in the sera of animals which were rendered tolerant by large intravenous doses of antigen followed by the administration of nose drops. The antibody class of the serum antibody was in all cases predominantly of the

TABLE II

The Specific Antigen-Binding Characteristics of the γA Immunoglobulin Fraction of Serum and Bronchial Washings

Guinea pigs	γA units/ml		μg antigen specifically bound/100 units γA		Percentage total specific antigen binding attributable to IgA fraction*	
	Serum	Bronch. wash‡	Serum	Bronch. wash	Serum	Bronch. wash
Normal	100§	4.6	<5	<5	—	—
Footpad immunized‖	102	5.2	14.5	233	14	72
Nose drop immunized‖	98	5.0	17	118	13	62
Tolerant animals‖ Nose drop immunized	100	5.8	<5	50	—	60

* Calculated as: $\frac{\mu g \text{ antigen/ml bound to immunoglobulin}}{\text{total } \mu g \text{ antigen/ml bound to immunoglobulin}} \times 100.$

‡ Compared with respect to the normal serum standard.

§ Arbitrarily assigned to the concentration in a pooled sample of 10 unimmunized sera.

‖ For schedules see Table I and text.

IgG type, and there was no significant difference between antigen-binding capacity of the γ1 and γ2 globulin fractions.

In contrast to the serum where only 13–14% of the total antigen-binding activity was attributable to the γA fraction regardless of the immunization regime, the bronchial washing fluid contained predominantly antibody of the IgA type. 72% of the total antigen binding of bronchial secretions was due to this class after subcutaneous immunization and 62% after local (nose drop) application of antigen.

Animals given 5 mg DNP-HGG intravenously on the same day as nose drop immunization showed much lower antibody responses in their bronchial washings (Tables I and II). The response that was observed consisted of predominantly IgA antibody.

Subcutaneous immunization gave rise to a much higher specific antibody activity in serum (approximately 100 μg antigen bound/mg IgG) than in the

bronchial washings (about 20 μg antigen/mg IgG). Nose drop immunization resulted in a relatively much higher specific response locally (20 μg antigen/mg IgG compared with 15 μg antigen/mg IgG in serum). Similarly, the antigen-binding activity associated with the IgA fraction of the two fluids was 1:1.6 (serum:bronchial washing) after subcutaneous immunization and 1:7 after nose drop immunization.

Cell-Mediated Immune Response.—Splenic lymphocytes obtained between 7 and 26 days after subcutaneous immunization invariably caused inhibition of the migration of normal peritoneal exudate cells in the presence of DNP-HGG (Fig. 1).

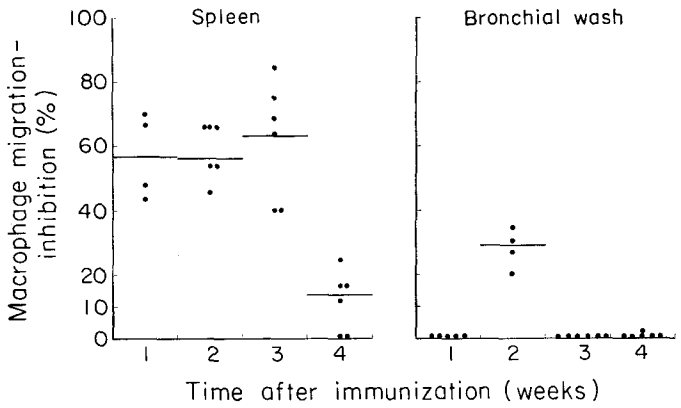

FIG. 1. Per cent inhibition of macrophage migration at various times after immunization of guinea pigs with DNP-HGG into the footpads. Each point represents one determination of migration inhibition. The horizontal lines represent the mean inhibition. Cells were obtained from the spleen and bronchial washings of the same animals.

Inhibition was demonstrable at 7 days, with peak levels of around 70% inhibition being observed at 19 days; far less inhibition was observed at 26 days. On the other hand, lymphoid cells obtained from bronchial washings from these same animals exhibited little or no inhibition of macrophage migration in the presence of antigen (Fig. 1). Differences in the macrophage migration-inhibition index between the splenic lymphocytes and the bronchial washing lymphocytes obtained 19 days after subcutaneous immunization were statistically highly significant ($P < 0.01$).

In the animals which were immunized by nose drops alone, a very different pattern was observed. After this immunization regime, splenic lymphocytes, when added to normal peritoneal exudate cells in the presence of antigen, showed virtually no inhibition of macrophage migration at any time after immunization (Fig. 2). However, lymphoid cells obtained from bronchial

washings of these animals 7 and 15 days after antigen administration strongly inhibited the migration of normal macrophages in the presence of DNP-HGG. This inhibition was, however, markedly decreased by 19 days and there was no inhibition at 26 days (cf. Figs. 1 and 2). Thus, systemic cell-mediated im-

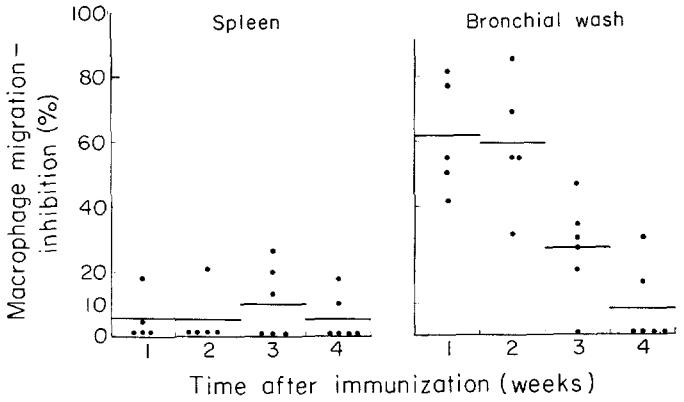

FIG. 2. Same as Fig. 1 except animals were immunized by nose drops with DNP-HGG.

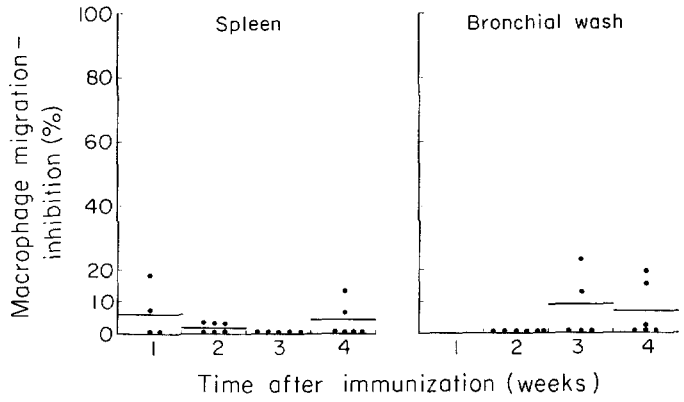

FIG. 3. Same as Fig. 2 except immediately before immunization by nose drops the animals were given 5 mg of DNP-HGG intravenously.

munity was induced after the subcutaneous route of antigen administration but not after nose drop administration of antigen. On the other hand, nose drop immunization gave rise to a significantly greater local cell-mediated immune response than did the parenteral administration of antigen.

In those animals which were immunized by nose drops immediately after a tolerizing dose of antigen intravenously, there was virtually no inhibition of

macrophage migration by either the cells from the spleens or bronchial washings (Fig. 3). Thus the induction of systemic tolerance markedly suppresses the local cell-mediated immune response.

Studies Delineating the Nature of the Macrophage Migration-Inhibition Activity of Bronchial Washing Cells.—Differential counting of bronchial washing fluid showed that approximately 70% of the cells obtained by the washing procedures employed were macrophages, the remaining 30% were designated by gross morphology and lack of carbon phagocytosis as lymphocytes. Using a trypan blue exclusion test, greater than 90% of the cells aspirated were shown to be viable. In order to determine whether the macrophage population from

TABLE III

Inhibition of Macrophage Migration by Purified Bronchial Washing Lymphocytes

Source of cells		Macrophage migration-inhibition*
Whole bronchial washing cell population‡	Immunized by nose drops	66 (±11)
	Unimmunized	2 (±0.6)
Purified bronchial washing lymphocytes§	Immunized by nose drops	73 (±16)
	Unimmunized	4 (±1.2)

* Mean of six determinations (± SEM).

‡ All of the cells obtained from the bronchial washings of three guinea pigs.

§ The lymphocytes obtained from the bronchial washings of three guinea pigs were purified by serial passage on glass Petri dishes, thereby removing the alveolar macrophages. Normal guinea pig peritoneal exudate cells were then used as the indicator cells in the macrophage-migration test.

immunized animals played an active role in the macrophage migration-inhibition activities of bronchial washing cells, the cells from an animal immunized by nose drops were sequentially transferred to a series of three glass Petri dishes, with an incubation period of 1 hr at 37°C between transfers. The resulting nonadherent cell population was morphologically greater than 99% lymphocytes. These lymphocytes were then mixed with peritoneal exudate cells from normal guinea pigs and this mixture was tested for the ability to inhibit macrophage migration in the presence of the antigen DNP-HGG. The results indicated that the lymphocytes alone from the bronchial washings were capable of inhibiting normal macrophage migration (Table III) and that there was no evidence of an enhancing effect due to "immune" macrophages.

To determine whether humoral antibody played a role in the macrophage inhibition shown by bronchial lymphocytes, the following experiment was devised. 10 million bronchial washing cells from a normal unimmunized animal were mixed with 5 ml of cell-free supernatant from a bronchial washing, the cells of which had previously been shown to inhibit macrophage migration in

the presence of antigen. The cell-free supernatant fluid was also shown to contain antibody, specifically binding approximately 50 μg/ml of DNP-HGG. This mixture of normal cells plus antibody containing supernate did not inhibit the migration of normal peritoneal exudate cells in the presence of DNP-HGG. Thus, the presence of antibody in bronchial washings does not appear to influence the performance of such washings in assays for the specific inhibition of macrophage migration.

In order to further delineate the nature of macrophage migration-inhibition by bronchial washings, 10^7 bronchial washing cells (containing approximately 7×10^6 macrophages and 3×10^6 lymphocytes) from an animal immunized with 200 μg DNP-HGG in nose drops 12 days previously were incubated with

TABLE IV

Production of Migration-Inhibitory Factory by Bronchial Washing Cells

Supernatant of incubation mixture of		Macrophage migration-inhibition*
Bronchial washing cells	Antigen	
Immunized animal	DNP-HGG	42 (±9.1)
	0	4 (±0.9)
Unimmunized animal	DNP-HGG	0 (±0.3)
	0	2 (±0.4)

* Mean of six determinations (± SEM).

300 μg DNP-HGG for 24 hr at 37°C. The cell suspension was then centrifuged at 1500 rpm for 5 min and the supernatant fluid was mixed with normal peritoneal exudate cells. The control was included in which bronchial washing cells from unimmunized animals were handled in the same manner and their supernatant fluid was mixed with normal peritoneal exudate cells. The results (Table IV), which show inhibition by supernatant fluid only from the immunized animals, indicate that a material which is similar to macrophage-inhibition factor (MIF) was liberated by the bronchial washing cells on incubation with homologous antigen.

DISCUSSION

This study was undertaken to compare three aspects of the immune response: (*a*) the effect of immunization on the development of the cell-mediated response on a secretory surface as compared to the systemic cellular response; (*b*) the cell-mediated as compared to the humoral antibody response with respect to route of immunization and local *versus* systemic response; and (*c*) the effect of induction of tolerance on the local humoral and cell-mediated immune response.

Bronchial washing cells from animals specifically immunized with nose drops

were shown to inhibit the migration of normal peritoneal exudate cells in the presence of homologous antigen. It is generally accepted that macrophage migration-inhibition is an in vitro correlate of cell-mediated immunity (14). This study presents data which indicate that inhibition of macrophage migration by bronchial washing cells should be similarly considered. Data is presented indicating that a substance analogous to MIF was produced by bronchial washing cells on incubation with homologous antigen, that the critical cell for production of MIF was the bronchial wash lymphocyte and not the macrophage, and that secretory antibody when mixed with normal bronchial washing cells could not induce the inhibition phenomenon.

This study supports the concept (6) that local cellular immunity can be initiated independently of systemic cellular immunity. In this study, guinea pigs which were immunized subcutaneously developed systemic cellular immunity in the absence of a significant response in their respiratory tracts. On the other hand, guinea pigs which were immunized locally developed local cellular immunity but no systemic cell-mediated immunity. This is an interesting phenomenon, since it contrasts with the classical concept of Gowans and Knight (15) that a very mobile, small lymphocyte population is responsible for this arm of the immune response. These results would suggest that at least in the respiratory tract there are lymphocytes which are locally sensitized and which remain in the area for several weeks. Systemic "sensitized" lymphocytes would appear not to migrate into the respiratory tract in significant numbers for a similar length of time. Whether the cells originate in the lamina propria of the bronchus and alveolar tissue or whether they come from local nodes is at present uncertain, but preliminary evidence (Henney, C. S., unpublished work) seems to suggest that the active cells are not seeded from local lymphoid tissue, but probably do come from the lamina propria. Comparable studies on other secretory surfaces such as the gastrointestinal tract have not been reported. Another study, however, has recorded that the relative independence of local and systemic cellular immunity can be overcome by large doses of antigens given either subcutaneously or locally[2].

The practical importance of the phenomenon of cellular immunity in the upper respiratory tract is emphasized by a recent report (16) in which protection against *Mycobacterium tuberculosis* entering through the respiratory tract was more closely related to cellular immunity in the tract than with systemic cellular immunity. Thus, it would appear that in infections of secretory surfaces in which cellular immunity may play a protective role, local immunization may be the route of choice. For example, studies on the protective effect of the

[2] Waldman, R. H., and J. E. Johnson, III. Cell mediated immunity demonstrated by lymphocytes obtained from bronchial washings. Presented at Reticuloendothelial Society Meeting, December, 1970.

aerosol administration of Bacillus Calmette-Guerin vaccine should be of great interest. Such studies are currently in progress.

Immunoglobulin levels in guinea pig bronchial washings indicate a relative increase in IgA as compared with the serum immunoglobulin levels. This is in agreement with numerous studies of external secretions in humans, rabbits, cows, pigs, dogs, etc. Besides this study, bronchial washings have been examined only in humans where it has been found that IgA predominated over IgG in human bronchial washings (17). The lack of isolated guinea pig IgA preparations make such comparisons on guinea pig bronchial washings impossible at this time. Furthermore, our measurements of the IgA in bronchial secretions were determined in relationship to a serum IgA standard. In human studies the difference between the levels obtained when a serum *versus* a secretory IgA standard are used is about 3–4-fold (1). In addition to the likelihood of errors arising in computation of the absolute levels of IgA in bronchial washings by using a serum standard, it should also be borne in mind that the relative antigen-binding properties (on a weight basis) between the IgA of serum and bronchial washings might also be expected to differ.

It is clear that differences between the systemic and the local antibody responses are associated with the routes of immunization. The immunoglobulin class of the antibody in serum was predominantly IgG whereas it was IgA in the bronchial washings. As has already been pointed out the specific activity of the various immunoglobulin classes was higher in serum (as compared to bronchial washing) after subcutaneous immunization, whereas after local immunization there was clearly a preferential increase in antibody formation in the respiratory tract. When compared to the cellular immune response, however, it would appear that the humoral response showed less independence of the local from the systemic response. The magnitude of the local antibody response to systemic immunization was a bit surprising, since numerous other studies have indicated that subcutaneous immunization does not stimulate high levels of local antibodies (2). Nevertheless, there have been studies in which systemic immunization has been shown to stimulate a humoral response locally (18). These differences are most likely accounted for by factors such as the quantities of antigen employed and their physicochemical characteristics.

It has been repeatedly shown in the past that marked suppression of the immune response can be induced in guinea pigs by the intravenous administration of large doses of antigen (19). This tolerance has generally been measured with respect to the development of serum antibodies, although delayed hypersensitivity skin testing has also been employed to study the development of cell-mediated immunity (20), and the suppression of MIF production by peritoneal exudate lymphocytes after a large dose of intravenous antigen has recently been demonstrated (21). The present study has investigated two other aspects of tolerance, i.e., the effect of the induction of systemic tolerance on the local antibody response, and on the local cellular immune response. The

results suggest that both the systemic and local antibody response are markedly suppressed after suppression of the immune response by a large intravenous dose of antigen. Similarly, the intravenous administration of large doses of antigen suppressed the development of sensitized lymphocytes in the bronchial tract in response to locally administered antigen.

SUMMARY

The cell-mediated immune response on the secretory surface of the lower respiratory tract was investigated and compared to the systemic cell-mediated immune response and to the secretory and systemic antibody responses. Guinea pigs were immunized either parenterally or locally with dinitrophenylated human IgG. The effect of rendering animals tolerant on the local cell-mediated response and on the local antibody response was also studied. The antibody response in the bronchial washing fluids was predominantly of the IgA class. In the animals rendered tolerant by administering large doses of the antigen intravenously, the secretory antibody response was significantly reduced to about 40% of the levels in the animals which were not tolerant. Cellular immunity was assayed using the inhibition of macrophage migration. In animals which were immunized subcutaneously, splenic lymphocytes strongly inhibited the migration of normal guinea pig macrophages; however cells obtained from bronchial washings from the same animals exhibited little or no inhibition of macrophage migration in the presence of antigen. In the animals which were immunized by nose drops, splenic lymphocytes showed virtually no inhibition of macrophage migration; however, bronchial washing lymphocytes strongly inhibited the migration of normal macrophages. Thus, nose drop immunization gave rise to a significantly greater local cellular immune response than did parenteral immunization. In those animals which were immunized by nose drops after a tolerizing dose of antigen, there was virtually no inhibition of macrophage migration by either the cells from the spleens or bronchial washings. Studies were done which demonstrated that a substance similar to macrophage-inhibition factor was liberated by the bronchial washing cells on incubation with the antigen. Studies were also done which indicated that secretory antibody played no role in the inhibition of macrophage migration when bronchial washing lymphocytes were used. Lymphocytes were purified from bronchial washings and were found to inhibit normal macrophage migration in the presence of antigen to the same extent that the mixed population of lymphocytes and pulmonary macrophages did. Thus this study supports the concept that local cellular immunity can be initiated independently of systemic cellular immunity.

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