investigation is the difference in urinary F.S.H./L.H. ratio between patients with a 47,XYY karyotype on the one hand and normal males on the other. Whereas the output of F.S.H. in the three subjects reported here was substantially normal, L.H. excretion was considerably increased. Most of the figures are in the range generally encountered in menopausal or postmenopausal women (*see* Papanicolaou et al. 1967). In normal males Becker and Albert (1965) cited a mean F.S.H./L.H. ratio of from 1 to 2; the corresponding ratio in the present investigation was 0·19.

It is remotely possible that the high L.H. values in the 47,XYY males as compared with the normal subjects of Becker and Albert (1965) could be explained on methodological grounds, since the assay procedures used in the two investigations (the hypophysectomised-rat-prostate test by Becker and Albert and the O.A.A.D. method in the present investigation) were different. However, such an explanation appears to be most unlikely in view of the observation of Papanicolaou (1968) that, when parallel assays by the 2 techniques are performed on urinary extracts prepared by the tannic-acid procedure of Johnsen (1958), similar results are obtained.

The reason for the abnormality in the differential excretion of F.S.H. and L.H. in males with a 47,XYY karyotype remains obscure. Findings on testicular biopsy might well throw some light on the question, and such data are available in at least two subjects with this sex-chromosome complement (Balodimos et al. 1966, Nielsen et al. 1966). The appearance of the testes was found to be similar to that encountered in classical cases of Klinefelter's syndrome, but not identical. Microscopically they were characterised by virtual absence of spermatogenic and Sertoli cells with relatively normal numbers of Leydig cells, which were arranged in small clusters and showed moderate hyperplasia. On the basis of these results it might be postulated that in men with a 47,XYY karyotype the Leydig cells of the testes are relatively insensitive to stimulation by pituitary L.H., and, in order to overcome this refractoriness, abnormally large quantities of the hormone must be produced by the anterior pituitary. The normal reciprocal relationship existing between the pituitary and the gonads does not appear to operate in 47,XYY males, since an abnormally high L.H. output in these subjects is associated with urinary testosterone readings which are above the normal range (Ismail et al. 1968).

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References continued at foot of next column

Hypothesis

MEASLES AS AN INDEX OF IMMUNOLOGICAL FUNCTION*

Summary There are two distinct systems of adaptive immunity in the body: one, mediated by cells differentiated in the thymus (T.-D. immunocytes), is concerned with delayed hypersensitivity and homograft immunity; the other with immunoglobulin and antibody production, the cell lines concerned being differentiated in gut-associated lymphoid tissue (G.-D. immunocytes).

The whole process of the eruptive stage of measles and subsequent immunity is mediated by the thymus-dependent system.

For this reason:

(*a*) In agammaglobulinæmia, where the gut-associated-lymphoid-tissue system is completely non-functional, measles follows its normal course and gives rise to normal subsequent immunity.

(*b*) In cortisone-treated acute leukæmia where the thymus-dependent system is eliminated, measles takes on the form of a fatal giant-cell pneumonia without rash.

(*c*) Subacute sclerosing panencephalitis is a condition in which the T.-D. system has developed specific tolerance (non-responsiveness) to measles antigen while the antibody system remains specifically active.

INTRODUCTION

MODERN immunological theory is based essentially on experimental work on laboratory animals. In recent years it has become almost mandatory to use pure-line strains of animals and techniques have become increasingly elaborate. Such work has produced a rich harvest of results, but it has not diminished the importance of contributions at the clinical level. In this article I am concerned with the effects of measles-virus infection in normal individuals and in persons manifesting certain immunological anomalies. The picture which emerges strongly supports the growing opinion among immunologists that immune reactions can be divided into two distinct categories. These correspond in man to what was described initially in birds as thymus-dependent and bursa-dependent systems.

DIFFERENT SEQUENCES

Normal Measles

The general quality of measles infection in children is known to everyone, and the picture presented in the normal disease need only be briefly outlined.

Measles is highly infectious and is apparently spread by droplet infection, the virus being liberated into saliva, &c., from the enanthem on mucous membranes at the end of the incubation period. It has a long incubation period

* A special lecture to virology students in the School of Microbiology, University of Melbourne.

DR. PAPANICOLAOU AND OTHERS: REFERENCES—*continued*

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of 10–14 days during which the child shows no overt evidence of illness. The only positive evidence as to what is happening during the incubation period is the presence of multinucleate giant cells in various lymphoid tissues. These are characteristic of measles lesions both in vivo and in vitro. It is generally assumed that on the initiation of infection a minimal mucosal lesion occurs and the virus, perhaps transported intracellularly in a mobile cell, reaches a draining lymph-node. Here the virus multiplies and, again probably in association with lymphocytes, is moved freely through the lymphoid tissues of the body.

From the tenth day, virus becomes free in the circulation and localises at various points which become evident as the punctate lesions of the enanthem including Koplik's spots, and a day or two later the typical measles rash, the exanthem. A few days after the exanthem has appeared, antibody becomes detectable in the circulating blood, and concurrently virus is no longer demonstrable and the patient is non-infectious. Once a child has recovered from measles he is immune for life. In 1847, Panum published a famous account of measles in the Faroe Islands in which the disease had been absent for 60 years. When the epidemic had been through the islands everyone was infected except those who had been children exposed to the previous epidemic 60 years before. This rather clearly makes two points: (1) that immunity against measles is life-long and specific; (2) that once a person has recovered from measles he never sheds virus into the environment. I would make the obvious deduction that the virus has been eliminated, but some workers still prefer to believe that, in one way or another, measles antigen continues to be produced in the body for the rest of life.

The measles virus is now known to be one of the larger myxoviruses like Newcastle disease, mumps, dog-distemper, and rinderpest, its closest relationships being with the last two viruses. Intranuclear inclusions and a tendency to giant-cell or syncytium formation are the characteristic effects in tissue-culture. Much antigen is developed in infected cells, and this can be detected by the use of a strong measles immune serum labelled with a fluorescent dye.

Once the virus had become accessible to laboratory study it was found, as would be expected, that antibody was produced soon after the attack, rose to a high level, and after a moderate fall persisted in demonstrable amount for many years, perhaps for life. It seemed clear enough that antibody was what protected the measles convalescent against reinfection. Measles seemed to be the perfect textbook model of an infectious disease. Everybody who has not had the disease is susceptible, every infected susceptible comes down with more or less typical symptoms and thereafter is immune for life. Antibody persisted in circulation for many years, and administration of convalescent serum during the early part of the incubation period could prevent or greatly attenuate the symptoms of measles. It seemed self-evident that immunity was a function of the persisting antibody.

Measles in Individuals with Immunological Anomalies

One of the most disconcerting discoveries in clinical medicine was the finding (reviewed by Good and Zak [1]) that children with congenital agammaglobulinæmia, who could make no antibody and had only insignificant traces of immunoglobulin in circulation, contracted measles in normal fashion, showed the usual sequence of symptoms and signs, and were subsequently immune. No measles antibody was detectable in their serum.

In congenital agammaglobulinæmia there is no evidence of abnormality in the thymus or in the number of circulating lymphocytes. In some ways the converse of this condition can be seen in children with acute leukæmia under cortisone treatment. Here there is presumably a great depression of normal lymphocytic function, and such children are extremely susceptible to infections of any sort. Chickenpox can be fatal, and, in three cases diagnosed as measles by isolation of the virus, Enders et al.[2] observed a fatal giant-cell pneumonia without rash. There is no reasonable doubt that this represents the pattern of measles infection in such immunologically crippled children.

Subacute Sclerosing Panencephalitis

The third manifestation of measles infection is a rare condition which has only recently been elucidated. It is now commonly called subacute sclerosing panencephalitis (S.S.P.E.), but it has been known under many other names for years. Histologically it is characterised by intranuclear inclusions and by diffuse infiltration with cells mostly glial but including plasma cells. It is a disease of childhood two and a half times as common in boys as in girls, which usually presents as a slowly progressive diminution in intelligence and gradual involvement of other cerebral functions going on to amentia, spastic paralysis, coma, and death. There are occasional remissions.

In 1965 a French group of electron-microscopists, Bouteille et al.,[3] found that the structure of the inclusions closely resembled that of measles-virus inclusions, and early in 1967 Connolly et al.[4] found that in three cases of S.S.P.E. there was a very high titre of measles antibody in the serum and in the cerebrospinal fluid. It appeared that S.S.P.E. was a manifestation of measles. It is a very interesting sign of the times that, within weeks of Connolly's discovery, almost every laboratory in America where measles antibodies could be titrated was investigating the condition; on Sept. 13, 1967, there was a full-scale symposium at Bethesda and a special number of *Neurology* containing the papers was published in January, 1968.[5] There were 75 participants from America, 10 from Europe, and 2 from South America.

The work has been well done, and it is now clear that in a very minute proportion of children who have measles something unusual happens. A slow infection begins in the brain and proceeds inexorably, the virus spreading from one cell into the next, provoking a neuroglial and plasma-cell response. Both glial and neuronal cells contain virus particles and tubules demonstrable in electron-micrographs and antigen recognisable by immunofluorescence. The brain is in fact saturated with virus and with antibody and antibody-making cells. Despite many attempts the virus has not been isolated, almost certainly because of the presence of antibody, yet the process goes on to death. The patients on record have had measles 4–17 years before the neurological symptoms appeared.

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3. Bouteille, M., Fontaine, C., Vedrenne, Cl., Celarue, J. *Rev. neurol.* 1968, **113**, 454.
4. Connolly, J. H., Allen, I. V., Hurwitz, L. J., Millar, J. H. D. *Lancet*, 1967, i, 542.
5. *Neurology, Minneap.* 1968, **18**, no. 1, part 2.

Significance for Immunology

Here, then, we have a challenge to theoretical immunology. Measles infection in man has a sequence known to everyone in the normal child; it is not recognisably different in congenital agammaglobulinæmia. In acute leukæmia with corticosteroid therapy it takes the form of a fatal giant-cell pneumonia without rash, while in S.S.P.E. there is a slowly spreading measles-virus infection of the brain in which abnormally large and persisting antibody production has no effect in blocking the process.

I believe that an attempt to interpret these differences in the pathogenesis of measles may be an excellent way of introducing the current picture of immunological theory.

It is one of the signs of a rapidly advancing subject that theory never remains static. Ever since the bursa of Fabricius was shown to have an immunological function, it has been evident that the thymus was unlikely to be the only *primary* immunological organ in mammals; but the question of the site and function of the mammalian equivalent of the bursa still remains unsolved. A variety of indirect evidence, however, points almost conclusively in one direction, which was first clearly indicated by R. A. Good.

Subject always to the qualification that the current theoretical interpretation is over-simplified, the following outline is probably close to the truth.

The specific phenomena of immunity are mediated by a system of cells which it is convenient to call immunocytes. These can be defined as cells which can react specifically with a defined antigen, more strictly with an antigenic determinant. It has long been known that there are two important sorts of immunocyte: those responsible for the production of antibody and those concerned with the phenomena of delayed hypersensitivity—the Mantoux reaction for tuberculosis being the prototype. The present tendency is to make this differentiation into two systems more complete and functionally more important.

All immunocytes of whatever type are derived from stem-cells which are characteristically present in the bone-marrow, but which may well be present in smaller numbers in the circulating blood, in the spleen, and possibly elsewhere. One can regard these stem-cells as in direct line from the fertilised ovum, the zygote, and as never having had a specialised function. Modern genetic theory would hold that during the sequence of stem-cell replications after fertilisation a process of diversification of potential immune pattern takes place. At the Cold Spring Harbor Symposium on Antibodies in 1967 there was extensive discussion of possible mechanisms for such genetic diversification in somatic cells, but no firm conclusions were reached. There was, however, a general opinion that, by the time they are ready for differentiation, almost every stem-cell is the potential producer of a different immune pattern—if it is fated to be an antibody producer, the specificity of that antibody will differ from that produced by almost any other cell. These immune patterns, however, do not become functional, they are not phenotypically expressed until the cells differentiate. At differentiation the immune pattern is expressed as immunoglobulin; and immunoglobulin, or at least that portion containing the specific combining site with its immune pattern, is incorporated as a specific receptor in the cell which now becomes an immunocyte.

Until recently, it was widely thought that this differentiation of stem-cell to immunocyte took place in the thymus only. Now it seems likely that the thymus is only one of the sites. With reasonable certainty the other site of primary differentiation is the gut-associated lymphoid tissue (G.A.L.T.) which includes tonsil, Peyer's patches, appendix, and perhaps other areas. In all probability, local hormone concentrations play a major part in differentiation and it is still uncertain how many such differentiating hormones exist. For the present we can speak of thymus-dependent (T.-D.) immunocytes differentiated in the thymus, and G.-D. immunocytes differentiated in G.A.L.T. Biology is a rather soft-edged subject, and all the experiments on which these conclusions are based are hardly ever more than 90–95% positive in the sense of supporting the sharp differentiation of the two systems. We may well have to modify the story a little as more detailed experiments accumulate and speak of thymus-dependent immunocytes differentiated in the thymus or in any other region where, regularly or under special circumstances, hormonal and other conditions are appropriate for thymus-type differentiation. The conditions for the G. type differentiation may turn out to be much more complex.

T.-D. immunocytes are responsible for delayed hypersensitivity and homograft immunity. They do not give rise to plasma-cells and produce only minimal amounts of type M immunoglobulin. G.-D. immunocytes give rise to plasma cells producing IgM, IgG, or IgA antibodies. Possibly each type arises in response to a different hormone, but it seems more reasonable to believe that there is a common differentiating stimulus and that the A, G, or M choice is made by intrinsic genetic processes. Mitchell and Miller's [6] important discovery was to show that, for some types of antibody, production resulted only if thymus-dependent cells were specifically stimulated in the immediate vicinity of the G.-D. cells. My interpretation of this as a non-specific pharmacological stimulation [7] is not mentioned by Mitchell and Miller, but seems best to fit the facts—notably that a wide range of other antibodies do not need this cooperation of thymus-dependent cells.

Once the difference between thymus-dependent (T.-D.) and non-thymus-dependent (G.-D.) cells has been grasped it is easy to see their relevance to the measles situation. The whole pattern of measles pathogenesis and immunity is clearly based on T.-D. immunocytes; measles is in fact a complex and severe delayed hypersensitivity reaction. The G.-D. system and its antibody are side-effects, epiphenomena, of minimal or no importance.

PATHOGENESIS OF MEASLES

Let us use this approach first to interpret the pathogenesis of measles in the normal individual. A virus particle lodges perhaps in the lining of a bronchiole; it produces a microscopic local lesion; and descendant particles, perhaps carried on lymphocytes, pass to the draining lymph-node. To understand the behaviour of measles virus and the cells involved in human infection it it desirable to say something about cytological aspects. Measles is not a necrosing virus; proliferation takes place, inclusion bodies and antigen are produced, but the cell does not easily break down and liberate virus. Instead, it modifies the surface of the cell so that it can fuse with an adjacent cell and allow the passage of virus to another cell by a wholly intracellular route. In part this will result in the production of giant-cells, but we can be equally certain that in the actively motile populations of lympho-

6. Mitchell, G. F., Miller, J. F. A. P. *Proc. natn. Acad. Sci. U.S.A.* 1968, **59**, 296.
7. Burnet, F. M. *Nature, Lond.* 1968, **218**, 426.

cytes many will also be infected by transient contact and move on their way to other lymphoid organs. These will come to contain increasing numbers of measles cells, carrying antigen on their surface but not necrosing and giving rise to no symptoms.

While this accumulation of cells carrying measles antigens is going on, an occasional such cell will make contact with an immunocyte (T.-D.) which has a pattern reactive with measles antigen. This will stimulate the proliferation of such an immunocyte to form a clone of similar cells which will move to other lymph-nodes. In this way, two populations of cells build up in the body: (1) cells carrying measles antigens either as virus or as smaller units, and (2) thymus-dependent immunocytes potentially or actually capable of reacting with measles antigen; and at a certain stage, active interaction begins. The nature of the trigger can only be guessed at, but the result is clear. Reaction of specific immunocyte and measles cell takes on a damaging intensity with disruption of the measles cell and liberation of extracellular virus and antigen. Antigen can now be available to stimulate the proliferation of G.-D. immunocytes, while virus, some free but most still associated with mobile cells, passes into the blood to lodge in skin and mucous membranes. Sensitised lymphocytes (T.-D. immunocytes) are also liberated in large numbers into the circulation. They will tend to leave the blood in the skin capillaries and elsewhere to produce the equivalent of delayed hypersensitivity reactions wherever antigen is being produced or concentrated. This onslaught is apparently sufficient in itself to eliminate virus from the body within a few days. In the normal individual it will be greatly helped by the rapidly increasing antibody in the blood.

In the course of the generalised delayed hypersensitivity reaction which we see as the measles rash, there is discharge and exhaustion of all those local cells, probably including mast cells, which can contribute pharmacologically to the local reaction. We can also assume that the regions from which large numbers of T.-D. cells are liberated have been temporarily exhausted. Taken together these are responsible for the failure of the classical Mantoux reaction against tuberculin to be elicited in the weeks following measles. The phenomenon is a clear indication of the fact that the measles rash is itself a diffuse delayed hypersensitivity reaction.

There is a well-known loss of reactivity to tuberculin and similar tests in a variety of lympho-proliferative conditions in man, notably Hodgkin's disease. Acute leukæmia in children also crowds out from the lymphoid tissues most of the lymphocytes that are functionally T.-D. immunocytes. Cortisone damps down the function of those that remain. Giant-cell pneumonia merely means that, in the absence of a delayed hypersensitivity reaction directed against them, measles cells adhere to and infect others instead of breaking down and initiating the eruptive phase of the disease.

Interpretation of S.S.P.E.

The explanation of S.S.P.E. is more difficult and probably more interesting. Clearly, by hypothesis there is a failure of T.-D. immunocytes to mount any attack on the cells infected with measles virus. This cannot be based on any genetic deficiency of the T.-D. system because all the children affected have gone through a normal attack of measles years previously and there is nothing in their histories to point toward any other immunological anomaly. To account for the situation we must postulate a specific non-responsiveness (tolerance) of the T.-D. system with a normal antibody (G.-D.) response. Some acquired anomaly is needed which persistently prevents the recruitment of T.-D. immunocytes reactive against measles antigens.

A widely held interpretation of tolerance is that it represents an absence of specifically reactive immunocytes as a result of the presence of the antigen in the situation where stem-cells differentiate to immunocytes. The newly differentiated T.-D. immunocyte is unduly sensitive to the stimulus of antigenic contact and, if the antigen concerned is present in the thymus, the cell is actually destroyed. Most immunologists accept that, where there is classical tolerance to skin-graft from another strain, the animal concerned is a chimera in which donor cells are present in thymus and other lymphoid tissues.

As a working hypothesis that is immediately available for experimental test, one could postulate that the essential anomaly in the S.S.P.E. patient is that the conclusion of a measles attack finds occasional measles cells, presumably lymphocytes, lodged in immunologically privileged areas in the sense that cellular reaction against them is difficult or impossible. Two of these are the central nervous system and the thymus. In the normal individual, one assumes that any activation of one of these cells in the brain to a level threatening damage will be dealt with essentially by circulating T.-D. immunocytes. Cells in which virus is inactive could probably persist for years. If in the thymus there is a slow cell-to-cell multiplication of virus with a constant presence within the thymus of measles antigen, then by hypothesis there will be no recruitment of T.-D. immunocytes, and defence against any ill-effects of the virus in the brain will be the responsibility of the immunocytes that were present at the time of the primary measles. Once these have been reduced and eliminated by the ordinary non-specific losses of lymphocytes, then, if no more are being recruited and there are still potentially infectious measles cells in the central nervous system, the process of S.S.P.E. can develop.

The experimental implications of this hypothesis are:

(1) That S.S.P.E. patients would fail to react to any measles skin-test antigen which gave clear negatives in susceptibles and definite delayed-type reactions in persons who had had normal measles.

(2) That in patients with S.S.P.E. the thymus should contain evidence of measles cells either as giant cells or as cells which show specific immunofluorescent or electron-microscopic findings. In younger children a biopsy specimen could probably be obtained without the necessity of splitting the sternum.

There is one other phenomenon with some resemblance to S.S.P.E.—the immunological behaviour of rubella infection in the fœtus. It is now well known that babies with signs of rubella damage are born excreting virus, as judged by isolation from pharyngeal swabs, and with antibody which appears to be undoubtedly of fœtal origin. In these children the antibody persists but the excretion of virus lasts, at most, for a month or two only. Here we are dealing with less aggressive virus than measles but one which has probably the same general behaviour. A temporary infection in the antenatal and neonatal thymus would account for the situation, and again should in principle be verifiable by examination of necropsy or biopsy specimens.

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