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特 別 講 演 1 Special Lecture 1

Genetic Control of the Immune Response—An immunological circuitry governed by major histocompatibility and immunoglobulin genes—

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The immunological circuit is an idea where antigenic input sets in a train process of consecutive activations of cells that finally produce an output which neutralizes the initial input. Thus, the immune response is not a selection of a single isolated clone but an activation of a series of interacting cells. Hence, one of the major questions we are asking is how the cell can find the right cell type to interact with to complete the immunological circuit. This question is immediately related to the recognition of antigen by the cell together with the recognition of the other cell to be activated.

Let us assume that the initial input is received by T cells. It has been well documented that T cells recognize antigen in association with the products of the self major histocompatibility complex (MHC), which in fact makes them possible to find right cell type to interact with. At the first step of antigen recognition T cells can be activated only when antigen is presented on the self-cell surface. This has been shown both in the activation of helper and DTH (delayed type hypersensitivity) T cells and induction of cytotoxic killer T cells. It is believed that this initial mode of antigen-recognition determines the consequence of cellular events that lead to the mediator release, cytotoxic killing of target cells, help and suppression of the immune response. How such an ability of T cells to recognize self MHC products is dictated is one of the hot debates in current immunogenetics.

We have previously reported that the antigen-specific T cell factor (TsF) can suppress the *in vivo* and *in vitro* antibody responses only when the donor of TsF and responding strains share the identical I-J subregion of the H-2 haplotype. Using a variety of recombinant strains and their F₁ mice as producer and responder of the keyhole limpet hemocyanin (KLH)-specific TsF, we were even able to determine the haplotype origin of I-J subregion of given recombinants.

The obvious question is how then such a restriction is required. We have shown that the initial suppressor T cell (Ts_i) carries an I-J determinant which is associated with Ig heavy chain V region structure (V_H) lacking known constant Ig structure. A major cross-

reactive idiotype was also detected on suppressor T cells primed with certain antigens. TsF obtained from Ts₁ also consisted of I-J and V_H as distinct polypeptide chains. The factor acts only a minute population of T cells having special cell surface markers (Lyt-1⁺,2⁺,3⁺) to induce a new suppressor population termed as Ts₂. The acceptance of TsF by the latter cell type was blocked by the treatment of Lyt-1⁺,2⁺,3⁺ cells with anti-I-J antiserum. Since antigen is present in excess and a large number of antigen-binding B cells are also around, the restriction specificity imposed by I-J subregion was supposed to be crucial for TsF to select the right target cell type which recognizes I-J portion of TsF in addition to antigen.

Our circuit model of the specific suppression of antibody response was based on the fact that I-J subregion gene products expressed on different subsets of T cells serve as the restricting elements for the consecutive activation of a series of cells involved in the suppressor pathway. The demonstration that TsF has another polymorphic structure, IgV_H, prompted us to explore whether or not V_H also serves as an restricting element in the regulatory circuit.

Antigen-binding T cells specific for 4-hydroxy-3-nitrophenyl acetyl (NP) were isolated from C57BL/6 and B10·BR spleen cells primed with NP on nonimmunogenic carriers. A portion of NP-binding T cells carried the allotype-linked major crossreactive idiotype (NP^b) which was detectable by heterologous anti-id and monoclonal antibodies against NP^b idiotypes associated with the framework (group II) and antigen-binding (group I) structures of primary anti-NP antibodies (provided by Dr. M. Reth). None of the monoclonal against λ chain and Igh^b allotypes reacted with isolated NP-binding T cells. The staining of the cells with anti-id was inhibitable by NIP-BSA.

TsF from NP-binding T cells as well as from a T cell hybrid clone (7C3-13) made by fusion with BW5147 could suppress NP-specific antibody response. Furthermore, the product of 7C3-13 clone had a very high haptenated phage inhibition activity which was heteroclitic (examined by Dr. M. Cramer). There was an interesting genetic restriction in which either H-2 or V_H match was necessary to induce the suppression of anti-NP antibody response. No suppression was observed in both H-2 and V_H incompatible combinations. In the case of V_H compatible but H-2 incompatible combinations, the overall suppression was generally slight or even undetectable, which the expression of NP^b idiotype was selectively suppressed. These results suggest that there are at least two pathways in NP-specific suppression, in which either MHC or Ig gene products are the restricting elements. The former is *via* the activation of I-J compatible acceptor cells which finally lead to the overall suppression, while the latter is *via* the interaction with anti-idiotypic helper T cells required for the expression of major idiotypes. The superimposition of these two pathways makes up the circuitry of the idiotype and MHC governed regulations, and suggests that both antigen and idiotype 'presented' by TsF in association with I-J subregion

products direct the response toward the suppression by selecting two second cell types, *i.e.*, I-J recognizing antigen-specific acceptor T cells and anti-idiotypic T cells.

From above observations, I would propose following conclusions: 1) The genetic control of the immune response is at the level of immunological circuitry rather than clonal selection. 2) Immune response genes govern the restriction specificities either by MHC or Ig gene products. 3) Any aberrant interactions between the family sets of immunocompetent cells cause serious disturbances in the circuit that lead to the abnormal enhancement or suppression of the immune response.

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特 別 講 演 2 Special Lecture 2

Chimeric Animals as Tools in Genetic Studies

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All mammals are genetic mosaics or chimeras since mutation must occur in somatic cells throughout life. Early mutation in the embryo can lead to large clones of cells readily observed in the adult. Late mutation in the adult can be expressed in abnormal differentiation and proliferation that results in tumor cells (Markert, 1978). Gene regulation and gene expression in any cell are clearly functions of the cellular environment which is an expression of the phenotype of the cells comprising the environment. The phenotype of each cell in its turn is dependent upon prior interactions of the cell with the environment to bring about those specific patterns of gene activity that generate the cell's phenotype. Thus a sequence of reciprocal interactions between a cell and its environment occurs that drives the cell along a specific path of differentiation until stability is finally achieved in the fully differentiated tissues of the adult (*cf.* Markert, 1965).

This process of cell differentiation is exceedingly complex and subject to error at each step in the chain of reactions. The system is buffered against deviation and a certain amount of variation in response is compatible with viability but may lead to abnormalities in structure or function. Moreover, cell death frequently occurs during embryonic development and much of this death may represent failures in developmental interactions. A great deal of cell redundancy seems to be built into cellular programs of development, at least in vertebrates. Of course, some cell death occurring at particular locations in the embryo is an essential programmed part of normal development (Saunders, 1966).

The subtleties of cellular interactions in development are ultimately referable to the initial genetic make-up of the cells. Whether secondary changes in DNA occur as a general mechanism underlying cell differentiation is not known except in lymphocyte differentiation (Abelson and Butz, eds., 1980). Nevertheless the patterns of gene expression certainly change during development and such changes are the very basis of the many cell phenotypes found in metazoans. Although the members of a single metazoan species differ somewhat in the alleles at various structural gene loci these are probably not sufficient to account for the differences among individuals. These differences stem largely from the timing and amount of gene activity in cells during development and not from the qualitative differences in gene products. Thus we expect most of the differences among individuals, since they are inherited, to lie in the non-coding or "regulatory" DNA (Markert *et al.*, 1975)

Analysis of the interacting roles of phenotype, regulatory and structural DNA, and cellu-

lar environment in generating new cell phenotypes has been handicapped by the uniform genetic constitution of the cells of a single organism. Now, however, we can make artificial chimeras by aggregating embryos of different genotype to form a single individual (Mintz, 1971). Thus cells of different genotype confront one another during embryonic development. These chimeras have revealed a new dimension in cell behavior—namely, the competitive ability of cells of different genotype to differentiate in particular directions, to proliferate, and to migrate to their final locations in tissues. For example, mouse cells differing in the alleles at a single locus, W vs w , reveal great differences in the ability to differentiate into melanocytes or into sperm (Gordon, 1977). Rat melanocytes differing in alleles at the H (hooded) locus show great differences in rates of development and migration, and they produce quite different pigment patterns in the adult.

The genotypes relevant to the chimeras used to test the effects on development of alleles at the W locus can be recorded as follows: $CCww$ —black, $CCWw$ —black with white belly spot, $CCWW$ —black-eyed white, $ccww$ —albino but wild type at the w locus. Note that $CCWw$ cells are fully capable of producing a nearly normal coat color. Only white feet and a small belly spot indicate the $CCWw$ genotype in contrast to wild type $CCww$ that is solid black. Aggregating $CCWw$ embryos with albino embryos ($ccww$) led to the development of ruby-eyed white animals, $CCWw \longleftrightarrow ccww$, rather than to the expected animals with a mosaic coat color of intermingled patches of black and white as seen in black—albino ($CCww \longleftrightarrow ccww$) chimeras. These ruby-eyed whites showed a conspicuous mosaicism of the pigmented retina of the eye when viewed under a dissecting microscope, but all the fur was white. The result can be explained by a superior competitive ability of ww melanocytes in migrating from the neural crest (the site of origin of melanocytes) and in fully populating all the hair follicles, so as to exclude Ww melanocytes.

The handicap imposed by the W allele was also evident in the differentiation of gametes. Nearly all gametes were derived from the albino component of the chimera although a few did come from Ww cells. It should be noted that Ww animals are normally fertile, although WW animals are sterile. Except for melanocytes and gametes all other cell types examined (by means of isozymic markers) were derived from both component embryos to about the same extent. A similar behavior of melanocytes was observed in rat hooded $\longleftrightarrow$ black chimeras (Yamamura and Markert, 1980). The hooded pigment pattern stems from the behavior of melanocytes. These develop more slowly in the posterior regions and migrate only a short distance to produce a black band down the back of the rat while leaving the sides and belly unpigmented. The white hair follicles are devoid of all pigment cells and any recognizable precursors. In the hooded $\longleftrightarrow$ black chimeras black melanoblasts populate hair follicles until their quota of pigment cells is filled. When the supply of black melanoblasts is exhausted, any remaining hair follicles remain white. The size of the white belly spot is proportional to the fraction of melanoblasts in the neural

crest that are derived from the hooded component. When nearly all are derived from the black component then there is no belly spot. Thus, these chimeras allow us to place the responsibility for the hooded pigment pattern entirely on the pigment cell and not at all on other cell types such as those making up the hair follicle. Nevertheless, the behavior of these pigment cells is a reflection of their own genotype as expressed in accord with the cellular environment.

Chimeras can be used not only to permit cells of different genotypes to interact but they can also be used to rescue genotypes that by themselves cannot enable an embryo to develop to term. One such is a diploid $\longleftrightarrow$ tetraploid chimera (Lu and Markert, 1980). Tetraploids are never born alive but such cells aggregated with diploid cells in chimeras do survive and enable tetraploid cells to contribute to many tissues even though in a defective fashion.

Fifty percent of the chimeras are sex chimeras, being mixtures of XX and XY cells. These sex chimeras develop normally usually as phenotypic males (Mullen and Whitten, 1971). This means that cells possessing a Y chromosome initiate the development of a testis from the sexually undifferentiated gonad but in other respects XX and XY cells exhibit equal capacities to develop into cells of either sex, except for the gametes themselves. There is no sex reversal of gametes in mammals.

From these several examples it should be evident that chimeric animals are very useful tools in genetic studies and that they provide us with new insights into the developmental genetics of cellular differentiation.

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