jections of the enzyme in SI, retrogradely labeled cells in SII and in the motor cortex (area 4) appear in narrow vertical groupings, which may be single or multiple, depending on the size of the injections.

These observations provide further evidence that the sensory cortex can be considered as a mosaic of functional units based on afferent input. In the two types of afferent systems examined here, the columnar pattern probably reflects a precise topographic ordering of cells and their axons. The width of the columnlike bands of axons and terminals, and their distribution, when taken in conjunction with available physiological data (*2*), make it unlikely that each band represents a single somesthetic modality; however, they probably indicate the anatomical organization that underlies the cortical representation of both place and modality. The investigation also shows, with previous studies on the thalamic afferents (*7*), that the granular and supragranular layers are the sites of termination of all three major afferent systems. This points up an interesting duality in the organization of the cortex, for evidence is accruing that the efferent fibers to subcortical centers such as the thalamus, midbrain, pons, and dorsal column nuclei arise only in layers V and VI (*10*).

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Delayed-Type Hypersensitivity to Sheep Red Blood Cells: Inhibition of Sensitization by Interferon

Abstract. *Interferon, when given or induced 24 hours before contact of mice with sheep red blood cells, prevented sensitization, and no delayed-type hypersensitivity reaction could be elicited 4 days later, after challenge with the antigen, as shown by the absence of footpad swelling in treated animals.*

Interferon, originally discovered because of its antiviral properties, has more recently been shown to affect the immune system (*1*). Our group has been interested mainly in the effects of interferon on cell-mediated immune reactions, and we have previously reported that, in mice sensitized to picryl chloride or sheep red blood cells (SRBC), expression of delayed-type hypersensitivity (DTH) is significantly inhibited if the animals are treated with interferon on the day before or the day of challenge with the antigen (*2*). We believe that this phenomenon contributes to the frequently described inhibition of DTH which occurs as a result of virus infection (*3*).

In view of this very pronounced action of interferon on the efferent arc of the DTH reaction, it was important to determine whether interferon also had an effect on the afferent arc, that is, the actual sensitization. This was examined with two different approaches: induction of interferon with Newcastle disease virus (NDV) in congenic high and low interferon producing mice, and administration of exogenous interferon.

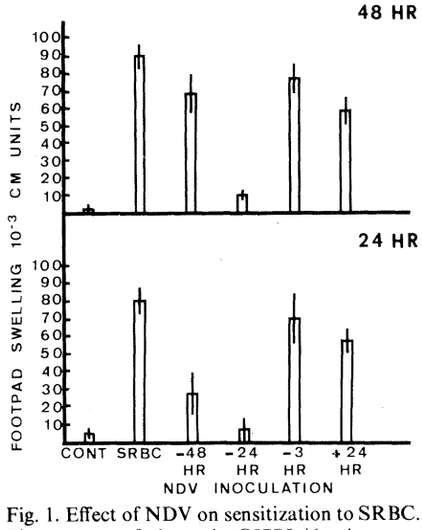

Fig. 1. Effect of NDV on sensitization to SRBC. Five groups of six male C57BL/6 mice were sensitized against SRBC. Three of these had been inoculated intravenously with 10^7 PFU of NDV, respectively 48, 24, or 3 hours before sensitization. The fourth group received NDV 24 hours after SRBC and the fifth group, which served as a sensitized control, received SRBC without any NDV injection. All mice, 4 days after sensitization, plus an additional group of unsensitized animals, were injected with 10^8 SRBC into the left footpad. Average footpad swelling (± S.E.) is graphically represented for each group 48 hours and 24 hours after sensitization. All values were virtually back to normal after 72 hours. Abbreviations: cont, unsensitized controls; SRBC, sensitized controls (SRBC without NDV injection).

The induction of DTH to SRBC in mice was carried out by the procedure of Lagrange *et al.* (*4*), in which mice are sensitized with an intravenous inoculation of 10^6 SRBC suspended in phosphate-buffered saline (PBS) and 4 days later, challenged by an inoculation into the left footpad of 10^8 SRBC suspended in 40 μl of PBS. Footpad swelling is measured with dial-gauge calipers 24, 48, and 72 hours later, and is expressed as the difference between the left (challenged) and the right (unchallenged) foot. Histological examinations made during previous experiments had shown that DTH reaction typically occurred 24 hours after footpad inoculation (*2*).

An experiment was carried out to determine whether interferon had any effect on sensitization to SRBC, and, if so, to determine the optimal conditions of timing. Different groups of six C57BL/6 mice received one intravenous inoculation of 10^7 plaque-forming units (PFU) of NDV, each group at a different time with regard to the time of sensitization, which was the same for all animals. The amount of virus inoculated was calculated to induce about 200,000 units (*5*) of circulating interferon at peak levels, that is, about 9 hours after virus injection. Footpad swelling, measured 24 and 48 hours after challenge, is illustrated in Fig. 1. All groups reacted to footpad challenge except the one that had received NDV 24 hours before sensitization, whose members behaved like nonsensitized animals. The fact that NDV had no effect when given on the day of or the day after sensitization was important, since it ruled out the possibility that the inhibition of footpad swelling in mice inoculated on the day preceding sensitization might have been due to an effect on the reactive or efferent phase.

That NDV acted through interferon induction was confirmed in congenic high and low interferon producers. Two strains of mice, genetically identical as far as all identifiable loci are concerned, but differing by their alleles at the If-1 locus, were used. One strain carries the If-1^h

(high producer) allele, and the other the If-1^l (low producer) allele (*6*). After NDV inoculation, circulating interferon titers at peak levels differ by about tenfold. In view of the results of the experiment described above, animals of the treatment group received NDV 24 hours before sensitization with SRBC. Challenge was given 4 days after sensitization, and the evolution of footpad swelling was recorded (Fig. 2). Inhibition of sensitization, as witnessed by absence or decrease of footpad swelling, was more pronounced in the high interferon producers than it was in the low producers, which provided direct evidence that the virus acted on sensitization through interferon induction.

Further evidence for the role of interferon was obtained in two experiments in which interferon made in tissue culture was administered to BALB/c mice 24 hours before sensitization. For one experiment, interferon prepared in C243 mouse cells with NDV as inducer was concentrated and partially purified by two consecutive steps of ammonium sulfate precipitation, as described by Tovey *et al.* (*7*). The specific activity of this interferon was 2.3×10^6 units per milligram of protein, and its titer was 1.4×10^6 units per milliliter. Mock interferon was prepared under identical conditions, except that the cells were treated with allantoic fluid instead of NDV. The purified interferon that was used in the second experiment was prepared in mouse L cells, with NDV as inducer, and was purified by affinity chromatography, by the method of Sipe *et al.* (*8*). The specific activity was 3.8×10^8 units per milligram of protein, and the titer was 2.8×10^6 units per milliliter. One percent of BALB/c serum was added to the preparation for stabilization. Mock interferon consisted of desorption buffer of a corresponding molarity, also enriched with 1 percent mouse serum. In both experiments two intraperitoneal inoculations of 1 ml of either interferon or mock interferon were given at a 5-hour interval, with the second inoculation given exactly 24 hours before sensitization. A third group of untreated animals was also included. Four days after their first contact with antigen all animals were challenged, and the evolution of footpad swelling was recorded during a 3-day period. The results, graphically illustrated in Fig. 3, show unequivocally that sensitization had been prevented in the interferon-treated mice.

Thus, in addition to its reported action on the efferent limb of DTH, interferon also has an effect on the afferent pathway of the DTH reaction. The absence of footpad reaction in the interferon-treated animals is not due to any residual effect on the

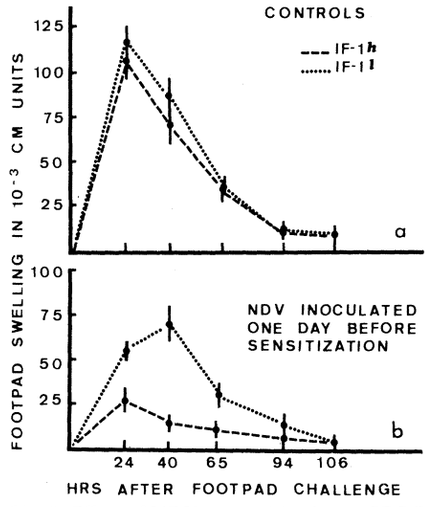

Fig. 2. Effect of NDV on sensitization to SRBC in If-1^l and If-1^h mice. The role of interferon in the inhibition of sensitization after NDV injection was examined in congenic high and low interferon producers. These mice received either allantoic fluid (as control) or NDV 24 hours before sensitization with SRBC. (a) Evolution of footpad swelling after challenge in control high (If-1^h) and low (If-1^l) interferon producers. In addition to serving as controls for the groups in (b), the results (a) show that mice of both strains react identically to SRBC sensitization. (b) Evolution of footpad swelling in both strains of mice when the animals have been treated with 2.1×10^6 PFU of NDV 24 hours before sensitization (this amount of virus induces peak levels of circulating interferon amounting to about 55,000 unit/ml in high producers, and 4,500 unit/ml in low producers). Sensitization was more efficiently inhibited in the high than in the low interferon producers. Results are means ± S.E. ($N = 6$).

efferent limb, as demonstrated by the timing experiment in C57BL/6 mice. Such a possibility is furthermore ruled out by previous results: in order to have an effect on the efferent limb, interferon or interferon-inducers must be administered on the day before or the day of challenge with hapten or antigen (*2*).

Increased DNA synthesis and blastogenesis of the lymphoid cells which drain the area in contact with the antigen represent the first events of sensitization (*9*); it may be that the antimitotic properties of interferon contribute to its effect on sensitization. In vitro interferon does inhibit blastogenesis in the mixed lymphocyte reaction, and in the mouse some interferon-inducers and interferon have been shown to decrease proliferation of cells derived from bone marrow (*10*). The recruitment of naive cells also intervenes in cell-mediated immune reactions, and there are clues, albeit controversial, that informational molecules, among which are messenger RNA's (mRNA's), are instrumental in this phenomenon (*11*). Translation of viral mRNA's is inhibited in interferon-treated cells, and an inhibitory effect of interferon on the translation of certain classes of cellular mRNA's can be envisaged, although no evidence for this hypothesis has yet been given (*12*).

Interferon represents one of the many lymphokines made by sensitized lymphocytes, and it may well be part of the regulatory mechanisms that control various aspects of DTH (*13*). However, interferon is distinguished from other lymphokines by the fact that it is made not only by specialized cells of the immune system, but also by any other cell of the body when properly induced. Of course, since the molecular identity of immune and nonimmune interferons has not been established, it may turn out that they differ considerably. In any case, interferon production represents, for nonimmune cells, a possible means of controlling certain functions of the im-

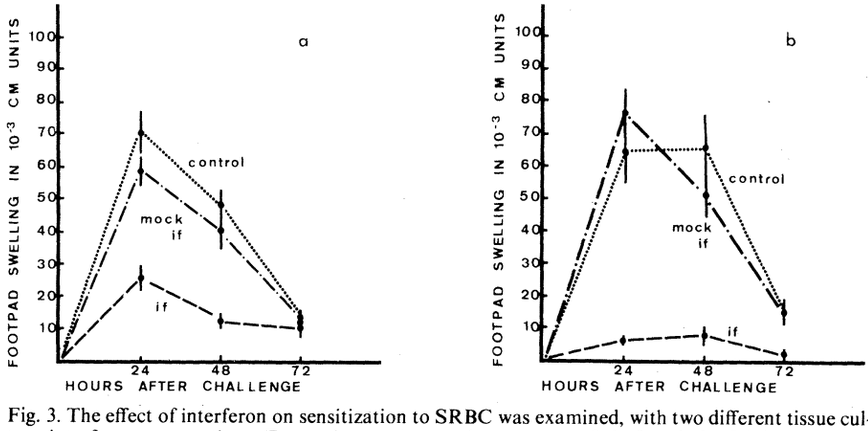

Fig. 3. The effect of interferon on sensitization to SRBC was examined, with two different tissue culture interferon preparations. For each experiment, three groups of six female BALB/c mice were used: 24 hours before sensitization with SRBC, one group was treated with interferon, one with mock interferon, and the third was left untreated. Evolution of footpad swelling was recorded, as measured on days 1, 2, and 3 after injection of the challenge into the left footpad. The mean values (± S.E.) are plotted in the figure. (a) C-243 cell interferon, semipurified by ammonium sulfate precipitation. (b) Highly purified L cell interferon.

mune system. The amount of tissue culture interferon purified by affinity chromatography that was used in our experiments totaled about 5×10^6 units per mouse, which corresponds roughly to 2×10^5 units per gram of tissue. This represents at the most 0.5 μg of protein per gram of tissue, which, on a weight basis, makes interferon a highly active inhibitor of cellular immunity.

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Synthesis of [6-^{3}H]-1α-Hydroxyvitamin D_3 and Its Metabolism in vivo to [^{3}H]-1α,25-Dihydroxyvitamin D_3

Abstract. *[6-^{3}H]-1α-Hydroxyvitamin D_3 was chemically synthesized and its full biological activity and radiochemical purity were demonstrated. With the use of this preparation it has been possible to demonstrate in vivo that in rats the [6-^{3}H]-1α-hydroxyvitamin D_3 is converted to [6-^{3}H]-1α,25-dihydroxyvitamin D_3, the natural hormone. In fact, in the intestine and bone of rats given 32 picomoles of [6-^{3}H]-1α-hydroxyvitamin D_3 each day for 6 days, more than 80 percent of the lipid-soluble radioactivity exists as [6-^{3}H]-1α,25-dihydroxyvitamin D_3, a finding that suggests that much of the biological effectiveness of 1α-hydroxyvitamin D_3 is due to its conversion to 1α,25-dihydroxyvitamin D_3.*

In order for vitamin D to carry out its physiological role in stimulating the mobilization of calcium from the intestine and bone, it must first be hydroxylated on C-25 in the liver and on C-1 in the kidney to 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$] (*1*). The fact that the kidney is essential in the synthesis of this hormone has led to the idea that renal osteodystrophy may be directly related to depressed production of 1,25-$(OH)_2D_3$ and that replacement therapy with the hormone would be useful in correcting these lesions (*2*). The first reported chemical synthesis of 1,25-$(OH)_2D_3$ suggested that synthesis of this hormone might be difficult and expensive (*3*). This prompted the synthesis of 1,25-$(OH)_2D_3$ analogs that were easier and less expensive to prepare (*4*). The most promising analog appeared to be 1α-hydroxyvitamin D_3 (1α-OH-D_3) (Fig. 1A), which was prepared as a model for the synthesis of 1α,25-$(OH)_2D_3$ (*5*). Holick *et al.* (*5*) showed that 1α-OH-D_3 is capable of stimulating intestinal calcium transport and bone calcium mobilization in normal and anephric rats. Furthermore, this analog is about 20 to 50 percent as active as 1,25-$(OH)_2D_3$ on a weight basis in the rat bioassay systems (*6*) and equally as active in eliciting biological responses in the chicken (*7*).

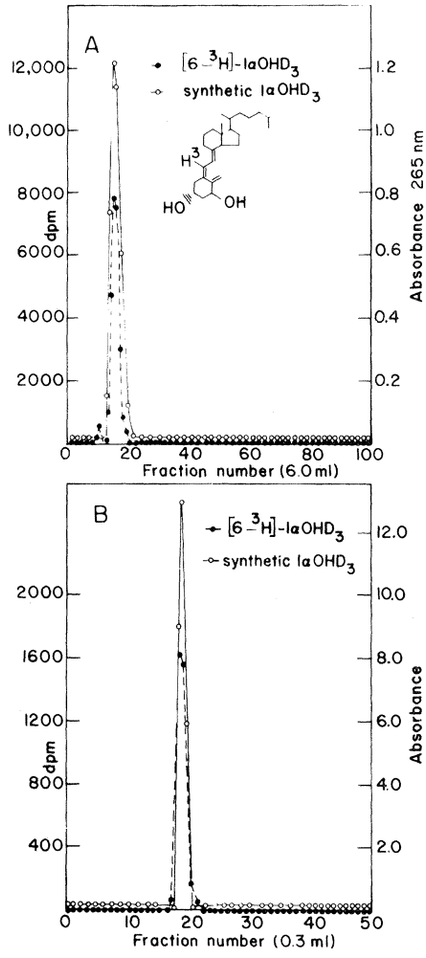

Fig. 1. Cochromatography of [6-^{3}H]-1α-OH-D_3 with crystalline synthetic 1α-OH-D_3. (A) Sephadex LH-20 column (1 by 60 cm) packed and developed with $CHCl_3$ and Skellysolve B (1 : 1). (B) High pressure liquid chromatography (2.5 cm by 2.0 mm) on Zorbax-Sil (du Pont de Nemours), developed with isopropanol and Skellysolve B (1 : 9). Abbreviation: *dpm*, disintegrations per minute.

The question as to whether 1α-OH-D_3 is hydroxylated on C-25 before it can function has been raised (*6–8*). Zerwekh *et al.* (*8*), using their chromatin-associated 1,25-$(OH)_2D_3$ receptor assay for 1,25-$(OH)_2D_3$, have suggested that the intestine of the chicken rapidly converts 1α-OH-D_3 to its 25-hydroxy derivative and that this hydroxylation can be demonstrated in vitro. However, as Zerwekh *et al.* stated, the metabolism of 1α-OH-D_3 to 1,25-$(OH)_2D_3$ can only be established with radioactive 1α-OH-D_3.

Using an intermediate available through our earlier work on the synthesis of 1,25-$(OH)_2D_3$ and 1α-OH-D_3 (*3*, *5*), we have prepared [6-^{3}H]-1α-OH-D_3 and have demonstrated that it is metabolized to 1,25-$(OH)_2D_3$. The introduction of a tritium at C-6 was accomplished by reducing 1α,3β-diacetoxy-5α-cholestan-6-one (*9*) with $NaBT_4$ (New England Nuclear) in isopropanol. The resulting [6-^{3}H]-6β-alcohol was dehydrated in pyridine with $POCl_3$ to yield [6-^{3}H]-1α,3β-diacetoxycholest-5-ene. Allylic bromination with *N,N'*-dibromodimethylhydantoin and dehydrobromination with trimethyl phosphite yielded the corresponding $\Delta^{5,7}$-intermediate (λ_{max} 295, 282, 271 nm), which on irradiation yielded 1α-[6-^{3}H] hydroxy-previtamin D_3-1α,3β-