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Bone Marrow Origin of Ia Molecules Purified from Epidermal Cells

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Using radiation bone marrow chimeras, we have shown that Ia molecules purified from epidermal cell preparations of the mouse reflect the Ia phenotype of the bone marrow donor. This result strongly suggests that Ia molecules are synthesized by a bone-marrow-derived cell in the epidermis. Furthermore, results of peptide map analysis of immunoprecipitated biosynthetically labeled Ia suggest that the Ia molecules found in skin are identical to those found on B lymphocytes. These results support biochemical as well as serologic identity.

The major histocompatibility complex of the mouse (H-2) has been the focus of extraordinary interest in the last few years. Since the description of Ia antigens, our group and others have investigated the role these determinants play in the regulation of immune responses [1–3]. Although the precise role of Ia antigens in immune responses is not yet clear, it has become clear that compatibility for *I*-region-coded genes is necessary for effective T cell-macrophage interaction. Furthermore, antisera directed against Ia antigens can inhibit immune responses requiring macrophage-T cell cooperation [4,5]. Thus, I-region antigens must play an important role in the regulation of immune responses.

Reports concerning the tissue distribution of Ia antigens have been confusing. It is clear that I-region determinants are expressed by most B cells and at least some macrophages and T lymphocytes [6–8]. Results of original studies, using cytotoxicity analysis, suggested that Ia was present on a majority of cells prepared from mouse skin [7,9,10]. Results of more recent studies using immunofluorescence suggest that the Ia-positive cells are restricted to the Langerhans cell population [11,12]. All of these studies are limited since the investigators could not be certain that the molecules detected on the surfaces of skin and Langerhans cells were identical to those expressed on the surfaces of B lymphocytes. We have found that the Ia antigens purified from skin cells are chemically indistinguishable from those of B cells. Using radiation chimeras, we have found that the Ia antigens recovered from skin cells are synthesized by a bone-derived marrow cell [13,14], almost certainly the Langerhans cell.

MATERIALS AND METHODS

Mice

All strains used in these studies either were purchased from The Jackson Laboratory, Bar Harbor, Maine, or were produced in our laboratories at the University of Southern California.

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Abbreviations:
NP-40: Shell nonionic detergent-P-40
SDS: sodium dodecyl sulfate
Tris: tris(hydroxymethyl)aminomethane

Antisera

All antisera were produced in our laboratories as described by David, Schreffler, and Frelinger [15]. Sera were produced in congenic mice with donor/recipient combinations designed to minimize or eliminate differences outside the *I* region. Sera were prepared by hyperimmunization with a mixture of spleen, lymph node, and thymus cells. (The precise donor:recipient combinations used are shown in the figure legends.) Animals were never sensitized with skin grafts. All sera were characterized by cytotoxicity on a panel of independent and recombinant strains and by absorption analysis. The monoclonal antibody (10-2.15) used was provided by the Salk Institute, from a line developed by Herzenberg [16].

Preparation and Labeling of Cells

Spleen cells were labeled after dissociation in Hank's balanced salt solution supplemented with 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid and 5% dialyzed fetal calf serum. ^{3}H-Tyrosine and ^{3}H-leucine were added at a concentration of 1 mCi/ml of medium. Epidermal cells were prepared by trypsinization as described by Scheid et al [17]. Spleen cells were labeled at a cell density of 2×10^7/ml for 5 hr; epidermal cells were labeled at 10^7/ml. After incubation with ^{3}H-amino acids, the cells were collected by centrifugation and lysed in 0.01 M tris(hydroxymethyl)aminomethane (Tris), 0.15 M NaCl, and 0.5% Shell nonionic detergent-P-40 (NP-40), pH 7.4. Debris was removed by centrifugation.

Lens culinaris *Affinity Chromatography*

Lentil lectin was prepared by the method of Hayman and Crumpton [18], and coupled to a cyanogen-bromide-activated Sepharose 4B. The NP-40 extracts were passed over the column, and the bound glycoprotein fraction was eluted with 0.1 M α-methylmannoside.

Immunoprecipitation

The glycoprotein fraction was incubated with antiserum for 2 hr and precipitated with fixed Staph A for 1 hr. The Staph A was washed 3 times, and the bound material was eluted with 2% sodium dodecyl sulfate (SDS), 2% mercaptoethanol, 50 mM Tris, pH 6.8. The sample was loaded on SDS-polyacrylamide gels. After electrophoresis, the gels were sliced in 1-mm sections, and the samples were eluted in 0.01% SDS and counted in a liquid scintillation counter.

Production of Radiation Chimeras

Chimeras were produced as described by Von Boehmer, Sprent, and Nabholz [19]. Briefly, host mice were irradiated with 950 R by means of a linear accelerator. Within 24 hr, they received intravenous injections of 2×10^7 α θ treated donor bone marrow cells. Animals were tested by hemagglutination, lymphocytotoxicity, or both after 3 mo to determine the origin of the hematopoietic cells. Only animals with complete donor repopulation were studied.

RESULTS

The Ia molecules immunoprecipitated from epidermal cells had properties identical to those isolated from spleen cells. Furthermore, both A and E regional molecules were expressed on these populations (Fig 1). The K transplantation molecule was similarly precipitated. Comparison of the tryptic peptide maps of ^{3}H-tyrosine-labeled spleen and epidermal cells showed no differences in the peptide maps of the I-A molecules (Fig 2). These molecules were not contributed by contaminating B lymphocytes. Rabbit anti-μ could not immunoprecipitate any newly synthesized μ chain. Epidermal cell cultures with added B cells yielded larger μ than Ia peaks. If significant Ia had been

contributed by the B cells, an Ig peak would have been apparent.

These results, which are consistent with other observations, demonstrate that Ia is expressed in epidermal cell preparations. They say nothing about the origin of the Ia-producing cells. We had noted a great deal of heterogeneity in earlier studies on the frequency of Ia-positive cells in epidermal cell preparations. The original report of Hammerling et al [7] mentioned 30%, and Klein et al [9] reported that essentially every cell in the epidermis was Ia-positive by cytotoxicity tests. Later reports stated that in human beings [20], guinea pigs [21], and mice [11] the *only* Ia-positive cells were Langerhans cells. Results of these studies, which used immunofluorescence techniques, suggested that only a small number of cells in the skin were Ia-positive. At about the same time, information about immune function of the Langerhans cell populations began to emerge [22]. We reasoned that if Langerhans cells played an important immune function as antigen-presenting cells, they were probably from a bone-marrow-derived stem cell (as are macrophages) and consequently that study of bone-marrow-repopulated radiation chimeras would provide a critical way to examine the origin of Ia-positive cells in the skin. This supposition led to several predictions. If the Ia in the epidermis was derived from epithelial cells, irradiation and repopulation would have no

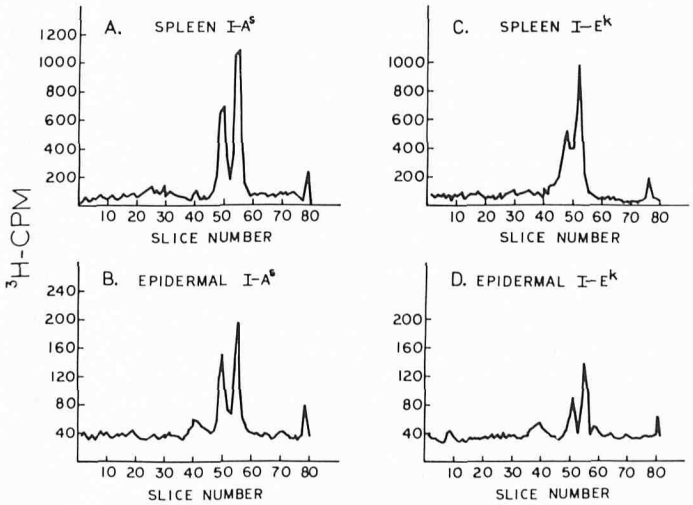

FIG 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of Ia molecules isolated by immunoprecipitation from B10.HTT mice. Serum A.TL anti A.TH was used to isolate I-A^s molecules and A.TH and anti A.TL was used to isolate I-E^k molecules. *A*, I-A^s from spleen. *B*, I-A^s from epidermis. *C*, I-E^k from spleen. *D*, I-E^k from epidermis.

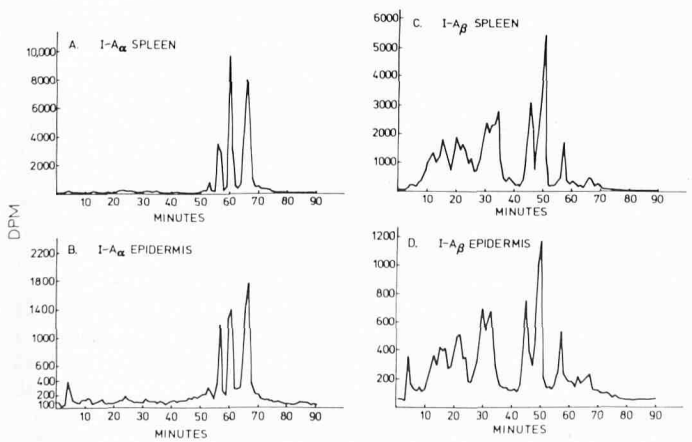

FIG 2. High-pressure liquid chromotography peptide maps of ^{3}H-tyrosine-labeled I-A molecules. *A*, I-A$^s_\alpha$ from spleen. *B*, I-A$^s_\alpha$ from epidermis. *C*, I-A$^s_\beta$ from spleen. *D*, I-A$^s_\beta$ from epidermis.

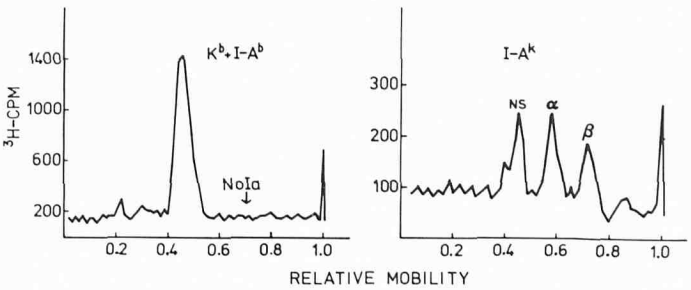

FIG 3. Sodium dodecyl sulfate-polyacrylamide gel patterns of Ia isolated from B10.A(1R) → [B10.A(1R)XB10]F$_1$ mice. *Left*, K^b but not I-A from the recipient. *Right*, I-A from the bone marrow donor. *NS*: a nonspecific peak, probably actin.

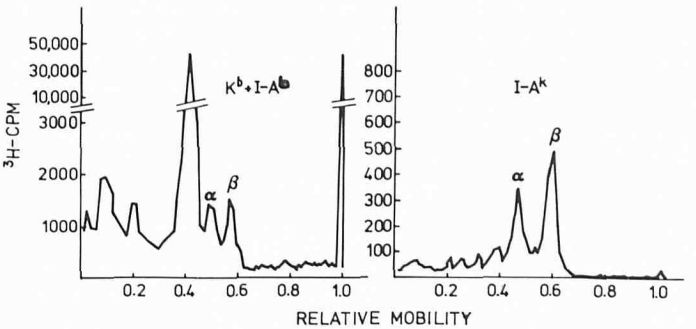

FIG 4. Sodium dodecyl sulfate-polyacrylamide gel patterns of Ia isolated from [B6XA]F$_1$ → B6 mice. *Left*, K^b and I-A from the recipient, from the bone marrow donor, or from both. *Right*, I-A^k, which can come only from the bone marrow donor.

effect on the Ia phenotype of skin in such animals. Thus, parent (P) → F$_1$ chimeras would express the F$_1$ Ia phenotype, and F$_1$ → P chimeras would express the parental type. On the other hand, if the Ia derived solely from Langerhans cells, such chimeras would express the reverse phenotype, that is, P → F$_1$ chimeras would express only a parental Ia molecule; an F$_1$ → P would express both parental types of Ia molecules. Intermediate results would also be possible, and would be detected as a quantitative variation in the concentration of each type of Ia. Therefore, we examined the Ia from tail skin preparations of P → F$_1$ and F$_1$ → P radiation chimeras. Figure 3 shows the results of P → F$_1$ chimeras; *only* Ia from the bone marrow donor used for reconstitution was detected, an indication that only the bone-marrow-derived cells provided the Ia precipitating from the skin. However, since the result was negative (ie., we did not detect the skin type), it was not by itself conclusive. The reciprocal experiment (F$_1$ → P chimeras; Fig 4) showed a direct positive result; both parental Ia haplotypes were detected by immunoprecipitation, direct evidence that the Ia was derived from a bone marrow cell. Detection of the K transplantation molecule in both experiments served as a control that non-bone-marrow cells were alive and producing appropriate membrane products.

DISCUSSION

Our results prove that the majority of Ia molecules detected by biosynthetic labeling and subsequent immunoprecipitation are derived from cells of bone marrow origin. These cells are almost certainly Langerhans cells. Two important *caveats* must be noted. First, this approach can never show anything but synthesis. Epithelial cells in skin might passively pick up Ia molecules that have been synthesized by Langerhans cells and then shed and passively adsorbed onto their membranes. Such passively acquired Ia might readily be detected under some conditions. The shedding and adsorption of molecules has been demonstrated recently by Emerson and Cone [23].* They

* Cone RE, personal communication.

showed that B cell Ia in the spleen can be shed and passively adsorbed by other cells. The second possibility is that non-bone-marrow-derived cells in skin might synthesize Ia themselves *de novo*; however, the turnover time of these cells in the membrane is much slower than in Langerhans cells. Consequently, the Ia produced by these cells has a lower specific activity and occurs in amounts so small as to be undetectable in immunoprecipitation analysis. Such conditions might be detectable by radioiodination experiments, but we have not examined this possibility. It is important to find out if Langerhans cells recirculate through the afferent lymphatics to draining lymph nodes and become the focus of germinal centers in these nodes. Some suggestive evidence for such recirculation has been recently reported [24]. An immune function is made even more likely by the demonstration of the bone marrow origin of the cells. We can now speculate that Langerhans cells, Kupffer cells of the liver, blood monocytes, and tissue macrophages are all part of the reticuloendothelial system and might represent simply different states of the same cell population.

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