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Epidermal Langerhans Cell-Targeted Gene Expression by a Dectin-2 Promoter

Makoto Bonkobara,* Paul K. Zukas,* Sojin Shikano,* Shinichiro Nakamura,† Ponciano D. Cruz, Jr.,* and Kiyoshi Ariizumi2*

Despite their critical function as APCs for primary immune responses, dendritic cells (DC) and Langerhans cells (LC) have been rarely used as targets of gene-based manipulation because well-defined regulatory elements controlling LC/DC-specific expression have not been identified. Previously, we identified dectin-2, a C-type lectin receptor expressed selectively by LC-like XS cell lines and by LC within mouse epidermis. Because these characteristics raised the possibility that dectin-2 promoter may direct LC/DC-specific gene expression, we isolated a 3.2-kb nucleotide fragment from the 5′-flanking region of the dectin-2 gene (Dec2FR) and characterized its regulatory elements and the transcriptional activity using a luciferase (Luc) reporter system. The Dec2FR contains a putative TATA box and cis-acting elements, such as the IFN-stimulated response element, that drive gene expression specifically in XS cells. Dec2FR comprises repressor, enhancer, and promoter regions, and the latter two regions coregulate XS-specific gene expression. In transgenic mice bearing a Dec2FR-regulated Luc gene, the skin was the predominant site of Luc activity and LC were the exclusive source of such activity within epidermis. By contrast, other APCs (DC, macrophages, and B cells) and T cells expressed Luc activity close to background levels. We conclude that epidermal LC are targeted selectively for high-level constitutive gene expression by Dec2FR in vitro and in vivo. Our findings lay the foundation for use of the dectin-2 promoter in LC-targeted gene expression systems that may enhance vaccination efficacy and regulate immune responses. The Journal of Immunology, 2001, 167: 6893–6900.

Abbreviations used in this paper: DC, dendritic cell; CS, consensus sequence; Dec2FR, 5′-flanking region of the dectin-2 gene; LC, Langerhans cell; Luc, luciferase; RLU, relative light unit; H-2RIIBP, retinoic responsive element in MHC class I gene; SVP, SV40 promoter.

Materials and Methods

Cell lines

XS106 and XS52 cells were obtained from A. Takashima (University of Texas Southwestern Medical Center, Dallas, TX). These cells are long-term DC lines established from the epidermis of newborn BALB/c mice maintained and expanded in complete RPMI 1640 supplemented with mouse rGM-CSF (1 ng/ml) and NS47 fibroblast culture supernatant (10% v/v) as source of CSF-1 (13). The NS47 line of dermal fibroblasts was maintained in complete RPMI 1640 supplemented with 10% FBS (13).
Ab and reagents

The mAb specific for CD8, Mac-1, MHC class II ε-18 (E9), CD45R, B220, CD3ε, CD11c, and FcR (CD16/CD32, FcyRII/III) were purchased from BD PharMingen (San Diego, CA). All chemical reagents used were obtained from Sigma-Aldrich (St. Louis, MO).

Cloning of Dec2FR and nucleotide sequencing

Genomic nucleotide sequences for dec2-2 were isolated from a genomic library prepared from BALB/c mouse (Clontech Laboratories, Palo Alto, CA) by hybridization with the cDNA for dec2-2 (Genbank accession number AF240357) (14). One of the isolated phage clones contained a 3.2-kb fragment of the 5′-flanking region that was amplified by PCR using Expand Long Template PCR System (Roche Diagnostics, Indianapolis, IN) with primers complementary to a 5′ end cloning site and a 3′ end sequence of 3′-untranslated region. The amplified nucleotide was subcloned into a plasmid vector, pGEM-7zf+ (pGEM-Dec2FR; Promega, Madison, WI), and its nucleotide sequence was determined at both sense and antisense strands by the automated sequencing of deletion mutants produced by Erase-a-base system (Promega).

Primer extension assay

This assay was used to map mRNA initiation sites on the 5′-flanking region. Fifty femtomoles of the synthetic oligonucleotide, 5′-CCAGAGTTTCGCAAATCACTCCACACATT-3′, was 5′-end labeled with [γ-32P]ATP and hybridized with 30 μg of total RNA isolated from X525 cells (10 μl; Superscript II Reverse Transcription System, Life Technologies, Grand Island, NY), the cDNA strand was extended from the primer toward the 5′ end. In the presence of the reverse transcriptase (10 U), Superscript II (Life Technologies, Grand Island, NY), the cDNA strand was extended from the primer toward the 5′ end of the 5′-flanking region. This extended strand was separated on 8% urea-PAGE in parallel with sequencing ladders (C, T, A, and G) synthesized from genomic DNA by the same primer. The location of the 5′ end (mRNA initiation site) was determined by the position of the nucleotide in the ladders showing the same size of the extended strand.

Construction of Luc expression vectors

The area containing a 5′-flanking region (nt –3176 to +1, designated the mRNA initiation site as +1) and the 5′-untranslated region was excised by digestion of pGEM-Dec2FR with MluI and XhoI restriction enzymes and introduced upstream a luciferase (Luc) coding sequence in pGL3-basic (Promega) without transcriptional control elements (pDec2FR-Luc). A second control was pGL3-control (pS40-Luc) (Promega), which contains SV40 promoter (SVP) and enhancer upstream and downstream, respectively, of the Luc gene. A set of deletion mutants lacking 5′-flanking sequences from its 5′ end was constructed by PCR-based mutagenesis. Briefly, a nucleotide fragment spanning the indicated 5′ end (Fig. 3) to the +126 was PCR-amplified and cloned into a plasmid-Basic Luc vector using MluI and XhoI sites. For deletion mutants designed to determine repressor and minimum promoter regions, a putative enhancer fragment (nt –2741 to –1850) was also PCR-amplified and inserted into some of the previous deletion mutants through SacI and MluI sites. For experiments examining whether the dec2-2 enhancer and a minimum promoter region (nt –123 to +126) control LC/DC specificity, we constructed a third set of Luc expression vectors. Dectin-2 enhancer and minimum promoter fragment were inserted separately or together into a pGL3-basic vector (pDec2E-Luc, pDec2P-Luc, and pDec2E/P2P-Luc, respectively). The enhancer fragment was also inserted into a pGL3-promoter (Promega) containing a SV4P sequence, using MluI and XhoI sites (pSVP/Dec2E-Luc).

DNA transfection and Luc assays

Luc vector DNA was delivered into various cell lines using liposome-mediated transfection (15). X5156 or NS47 cells, seeded on a 60-mm dish at a density of 1 x 10^6 1 day before, were cultured in the presence of 1 μg of DNA and 3 μl of FuGene (Roche Diagnostics). Following a 24-h incubation, whole cell extracts were prepared from cells by lysis in 1 X Retorter lysis buffer (Promega). For experiments in which tissue Luc activity was examined, proteins were extracted from excised tissues by homogenization in the lysis buffer (1 ml/100 mg of wet weight). An aliquot (10–20%) was used to measure Luc activities determined by light emitted for 30 s with an Optocomp I Luminometer (MGM Instruments, Hamden, CT) using Luciferase Assay System (Promega). Protein concentration was determined by the Bradford method, and their values were used to normalize Luc activity (16).

Electromobility shift assay

The nucleotide sequence –123 to –34 was segmented into three duplexed oligonucleotides: DM1 probe (35-mer), 5′-CCACATTTAGGAACCTGAGAGATTAAGAAACTTTGACCAAT (nt –123 to 89); DM2 probe (30-mer), ACACCTTCTCAGAGTCTTATGAAACAAT (nt –94 to –65); and DM3 probe (33-mer), AAATTTAGGTATTTTCTCAATTCCCCTT CGA, and 5′-CGGCGTCATCGTCGGGAAGA-3′ (34-mer). The oligonucleotide probe for pCAGG (22-mer; ATCTGATCTGAGCATGACGC) and a control competitor (GTTTGGCGCGGTATATCGGGAGCA) derived from the coding sequence for Luc version 3 (Promega) were also synthesized. These oligonucleotides were 5′ end-labeled with T4 DNA kinase and mixed with 10 μg of nuclear extracts. The preparation of nuclear extracts and electromobility shift assays were performed as described previously (17–18). For competition experiments, nuclear extracts prepared from X5156 DC were incubated with unlabeled DM2 or control competitor oligonucleotide at various concentrations before addition of radiolabeled DM2 probe.

Generation of transgenic mice

pDec2FR-Luc DNA was depleted of its plasmid sequence by digestion with MluI and BamHI restriction enzymes and was highly purified by agarose electrophoresis and DNA extraction from gel plugs using a Nucleo Spin Extraction kit (Clontech Laboratories). The purified DNA was microinjected into fertilized oocytes obtained from ICR mice (The Jackson Laboratory, Bar Harbor, ME) performed by the Transgenic Mouse Facility at University of Texas Southwestern Medical Center. Transgenic founders were identified by PCR analysis of genomic DNA (1 μg) extracted from tail biopsies using primers for Luc gene: 5′-GAAGTTCCGGGAAGGCT GTGCG-3′ and 5′-CGGCGTCATCGTCGGGAAGA-3′. After 30-cycle amplification, PCR products were separated on 1.5% agarose gel, transferred onto a membrane, and followed by Southern hybridization with the corresponding DNA probe. The founders whose tail DNA showed expected size of the PCR and specific hybridization were selected and examined for Luc activity in their tails. Consequently, three transgenic founders were generated, and two of them, showing the highest Luc activity, were selected as breeding partners. Their F2 offspring mice were produced by mating with ICR wild-type mice, and the mice aged 4–20 wk were used for experiments addressing the LC/DC specificity for Dec2FR promoter activity. Animals were housed in the pathogen-free facility of the Animal Resource Center at the University of Texas Southwestern Medical Center. All the experiments were conducted according to guidelines of the National Institutes of Health.

Depletion and purification of epidermal LC

Deletion and purification were performed using immunomagnetic beads. Epidermal cells were prepared from ear skins of eight transgenic mice, as described previously (19). Cells were processed in three ways: one batch (5 x 10^5 cells) was not treated, whereas the other batches (1.5 x 10^6 cells per plate) were incubated for 30 min on ice with 10 μg/ml rat mAb specific for CD8 (control) or MHC class II molecule (Ia). After extensive washing, cells were incubated with magnetic beads (2 x 10^5) coated with anti- rat IgG (Dyna Biotech, Lake Success, NY). Unbound (depleted of Ia^+ cells) and bound (purified for Ia^- cells) fractions were resuspended in 200 μl of PBS, and an aliquot (20 μl) was used for the cell counting. Protein was extracted from the rest of cells assayed for Luc activity using 4% of the total extract. The extent of deletion of Ia^- cells was estimated by reduction in frequency of Ia^- cells after treatment with the magnetic beads. Small aliquots of unfractinated epidermal cells (just before use of magnetic beads) and of a fraction not bound to anti-Ia were stained with 10 μg/ml FITC-conjugated anti-rat IgG. Untreated epidermal cells and cells of anti-CD8-unbound fraction were also stained with anti-Ia mAb. These stained cells were examined for frequency of Ia^- cells by flow cytometry.

Purification of leukocytes

DC, T cells, and B cells were purified from spleen. For DC, spleen cells were prepared from the same pool of transgenic mice as was used for preparing epidermal cell suspensions (Fig. 6). After pretreatment with anti-FcR Ab (10 μg/ml), the spleen cells (5 x 10^5 cells) were incubated with 10 μg/ml biotin-conjugated rat anti-CD11c mAb, followed by treatment with streptavidin-coated magnetic beads (5 x 10^5; Dyna Biotech). For T and B cells, after staining of spleen cells (2 x 10^6) with 10 μg/ml FITC-conjugated anti-CD3ε (145-2C11, Becton Dickinson) and 5 x 10^6 cells (CD19^+ B cells) were purified by flow cytometric sorting. For peritoneal macrophages, at day 4 after i.p. injection of 3% thiglycolate (in PBS), peritoneal cells were collected from the transgenic mice and cultured for 3 days in 10% FCS-RPMI 1640 (20). Following extensive washing of the culture dishes to remove floating cells and weakly adherent cells, peritoneal macrophages were harvested by scraping and examined for expression of Mac-1 and B220 to estimate contamination of peritoneal B cells (B220^+ ) using flow cytometric analysis. Typically, the adherent cells comprise <10% of B cells. Cell counting and Luc assays were performed as described above.

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6894 LC-TARGETED GENE EXPRESSION
Selective isolation of DC, macrophages, T cells, and B cells was confirmed by flow cytometric analyses and by parallel setting with control Abs.

Results

Dec2FR contains essential promoter and cis-acting elements

To isolate the transcriptional regulatory region of the dectin-2 gene, we screened a BALB/c mouse genomic library. Five independent phage clones were isolated, which cover ~40 kb of the dectin-2 gene containing six exons and a long stretch (3.2 kb) of Dec2FR (14). Primer extension assay and nucleotide sequence analyses revealed that Dec2FR has one major site for mRNA initiation at 126 bp from the initiation codon and a putative TATA box at 24 bp upstream of the initiation site (Fig. 1), both of which are essential elements for de novo mRNA synthesis.

The entire nucleotide sequence of Dec2FR was determined and a proximal region (nt −550 to +1) of the mRNA initiation site was searched for putative NF binding sites using a transcription factor II database. AP-1 binding sites (AP-1) (21), IFN-γ-responsive elements (22), retinoic responsive element (H-2RIIBP) (23), and an element (GM-CSF-consensus sequence (CS)) responsible for gene expression of GM-CSF by T cells in response to Ag stimulation (24) were found (Fig. 1B), suggesting that Dec2FR transcriptional activity may be up-regulated by a wide variety of stimuli, including phorbol esters, IFN-γ, retinoic acids, and Ag stimulation. We also noted the presence of an NF-Y-MHCII site, which controls MHC class II gene expression (25), suggesting that the Dec2FR may have a relationship with class II expression.

Dec2FR drives gene expression specifically in XS cell lines

Dec2FR or SV40 transcription units were linked separately to the Luc gene (pDec2FR-Luc and pSV40-Luc, respectively) (Fig. 2A) and introduced into various cell lines. pDec2FR-Luc produced 18-fold higher activity compared with pGL3-basic (promoterless Luc vector) in XS106 DC (Fig. 2B), a line that resembles epidermal LC phenotypically and functionally (13, 26). By contrast, the promoter activity was close to the baseline level in NS47 fibroblasts (Fig. 2B) and considerably lower or minimal in other cell lines including macrophage, B cell, T cell, and keratinocyte lines (48). The control SV40 transcription unit induced strong Luc activity in both XS106 DC (65-fold) and NS47 fibroblasts (153-fold higher than the baseline). This XS106 DC-selective expression of Dec2FR activity is consistent with our previous finding of exclusive dectin-2 mRNA expression in the same cell line (14).

Dec2FR consists of three functionally independent regions

To identify nucleotide sequences controlling Dec2FR promoter activity, we conducted deletion mutant analyses. Various nucleotide sequences were deleted from the 5′ end of Dec2FR (nt −3176 to +126), ligated to the pGL3-basic vector, and transfected into XS106 DC or NS47 fibroblasts (Fig. 3A). Dec2FR produced high levels of Luc activity (29 ± 6% of Luc activity by pSV40-Luc) in XS106 DC. Deletion of the region between −2741 and −1852 dramatically reduced transcriptional activity, suggesting that it is required for high activity, probably functioning as an enhancer. Progressive deletion within region −1851 to −507 partially restored activity, whereas further deletion of region −506 to +1 completely abrogated activity. To more precisely characterize region −1851 to −36, segments were progressively deleted and inserted into a Luc vector containing the putative enhancer fragment −2741 to −1852. Deletion of region −1851 to −507 markedly enhanced promoter activity (11-fold; 1681 vs 149 relative Luc activity). By contrast, further deletion of region −506 to −277 led to the lowest level of reduced activity shown by mutant −276. Surprisingly, mutant −123 produced increased activity, indicating the presence of a second repressor region between −276 and −124. Mutant −35, which contains a TATA box and its downstream region, displayed the lowest level of Luc activity (6% of Luc activity by pGL3-basic vector).

Progressive deletion within region −1851 to −507 resulted in increased Luc activity, suggesting that it is required for high activity. Finally, progressive deletion within region −506 to −277 produced variable Luc activity, indicating the presence of a second repressor region between −276 and −124. Mutant −35, which contains a TATA box and its downstream region, displayed the lowest level of Luc activity (6% of Luc activity by pGL3-basic vector).

FIGURE 1. Characterization of a proximal region of Dec2FR. A, Primer extension assay was used to map initiation sites for dectin-2 mRNA. Total RNA isolated from XS52 DC was primed for cDNA synthesis with 32P-labeled oligonucleotide, and the extended cDNA strand (cDNA) was separated on denatured PAGE in parallel with sequencing ladders (C, T, A, and G). An arrow indicates the 5′ end position of the cDNA strand (i.e., mRNA initiation site). A putative TATA box and the initiation site (designated as +1) are shown on the nucleotide sequence of Dec2FR. B, A proximal region (nt −550 to +1) of Dec2FR was searched for NF binding sites using a transcription factor database, revealing the presence of the following sites: AP-1 (12-O-tetradecanoylphorbol-13-acetate-inducible element), adenovirus-conserved (inverted terminal repeats in adenovirus promoter), BHLH-CS (consensus binding site for helix-loop-helix NF family), CCAAT/enhancer binding protein-TIRS2 (multiple elements in a promoter region of transthyretin), yIFN-CS (IFN-γ-responsive element in MHC class II gene promoters), GM-CSF-CS (responsible for GM-CSF expression by Ag- or mitogen-stimulated T cells), H2A-conserved (conserved site in multiple histone H2A promoters), H-2RIIBP (retinoic responsive element in MHC class I gene), IE1.2 (NF binding site in human CMV enhancer), NF-Y-MHCII (conserved sequence for class II gene promoters), and TATA box (essential promoter element).
region, lost almost all activity. When transfected into NS47 fibroblasts, all mutants produced considerably low activity ranging between 1.0 and 6.4 relative Luc activity to pGL3-basic. Because even mutant −123 retained selective expression in XS106 cells, we surmised that region −123 to +1 and the putative enhancer contain the minimal sequences required for the XS cell selectivity. Removal of a 5′-untranslated region (5′UTR), spanning nt +1 to +126, from the wild type and a mutant −123 reduced transcriptional activity by 72 and 20%, respectively. However, it should be noted that the 5′UTR itself did not produce any detectable Luc activity even in the presence of an enhancer (data not shown). These analyses indicate that Dec2FR consists of an enhancer (−2741 to −1852), two repressors (−1851 to −507 and −276 to −124), and a minimum promoter (−123 to +1) including a TATA box and an mRNA initiation site (Fig. 3C).

We next determined the role of the putative enhancer (Dec2E) and/or minimal promoter (Dec2P) in regulating the XS DC-specific expression. Dec2E fragment was inserted into a Luc vector with Dec2P or with the SVP and examined for Luc activity in XS106 DC or NS47 fibroblasts (Fig. 3D). Neither Dec2E nor Dec2P alone displayed strong activity in the XS DC, whereas activity was greatly enhanced when the two fragments were linked, with the level of enhancement much higher in XS cells than in fibroblasts. Moreover, Dec2E also enhanced the activity of the heterologous promoter, SV40, more intensely in XS cells than in fibroblasts (Fig. 3D). These data verify the presence of enhancer function in this region and also indicate that enhancer and minimal promoter coregulate XS DC specificity of dectin-2 gene expression.

Nuclear proteins that bind to the minimum promoter sequence are expressed abundantly by XS106 cells

Because a nucleotide sequence spanning −123 to −36 (88 bp) in the minimum promoter was shown to regulate transcriptional activity and selectivity of cell expression of the dectin-2 promoter, we performed electromobility shift assays to detect nuclear proteins that bind to the 88 bp. The sequence was divided into three regions: DM1 (nt −123 to 89), DM2 (nt −94 to −65), and DM3 (nt −66 to −34). DM1 probe detected several species of DNA-

FIGURE 2. Promoter activity of the Dec2FR fragment. A, Map of Dec2FR-driven Luc expression vector (pDec2FR-Luc). A 3.2-kb fragment isolated from Dec2FR contains a putative TATA box, a major mRNA initiation site (+1), and a full length of 5′-untranslated region (+2 to +126, shown by an open box). The filled box represents an SV40 late poly(A) signal. B, XS106 DC and NS47 fibroblasts were transfected in triplicate with pDec2FR-Luc, SV40 (enhancer and promoter)-driven Luc (pSV40-Luc), or Luc alone (pGL3-basic) and assayed for protein concentration and Luc activity (RLU). The Luc activity (1760 average RLU/μg) expressed by Dec2FR in NS47 cells was just above the baseline expression shown by pGL3-basic (1496 RLU).

FIGURE 3. Dec2FR contains three functionally distinct regions. A, Deletion mutants of Dec2FR were generated by PCR-based mutagenesis. 5′ end positions of mutants are shown by distance (bp) from the mRNA initiation site. Mutants were transfected into XS106 DC and examined for Luc activity. Transcriptional activity was indicated by the percentage of relative Luc activity to that of pSV40-Luc vector. B, Putative repressor and minimal promoter regions were mapped. A putative enhancer fragment (−2741 to −1852) was inserted downstream the Luc gene in deletion mutants of the region −1851 to −35 and measured for Luc activity in XS106 and NS47 cells. Each transcriptional activity was calculated as RLU of mutant relative to pGL3-basic. C, Data from two sets of deletion mutant analyses are summarized. Dec 2FR consists of, from the 5′ end, an enhancer, two repressors, and a minimal promoter including a TATA box and an mRNA initiation site. D, Enhancer and promoter components in the indicated Luc vectors are shown at the left; dectin-2 enhancer fragment (Dec2E), the minimal promoter (Dec2P), and an SVP. These Luc vectors were transfected into XS106 and NS47 cells and their transcriptional activities were compared with that of pSV40-Luc. These data (A, B, and D) were derived from triplicate assays, representative of three independent experiments.
protein complexes in nuclear extracts isolated from XS106 cells. Three of these species were also expressed in other cell lines (e.g., Raw macrophage, J558 B cell, BW5147 thymocyte, and S105 fibroblast lines) (Fig. 4A), whereas two minor species were expressed only by XS106 cells. However, the respective binding activities of these two latter species were not blocked with unlabeled DM1 oligonucleotide, indicating that binding was not specific (data not shown). DM3 probe detected six species, all of which were also widely expressed. Finally, DM2 probe detected four species in complexes formed with XS106 nuclear extracts, two of which (third and fourth species) were highly expressed in XS106 nuclear extracts, only minimally in macrophage extracts, and not at all in other cell extracts. The abundant expression in XS106 cells was not due to a higher concentration of nuclear proteins in the cells, because expression levels of complexes with DM1 probe and Sp1 transcription factor (Ref. 27 and data not shown) were similar for XS106 and Raw macrophage cells. Moreover, binding activity of XS106 nuclear extracts was almost completely inhibited by 50 ng of unlabeled DM2 (corresponding to a molecular excess of 1:300), but not by control oligonucleotide (Fig. 4B). These results indicate that the Dec2FR minimum promoter contains cis-acting element(s) that may play a role in regulating preferential expression of dectin-2 gene in XS cells.

**Tissue distribution of Luc activity in Dec2FR-Luc-transgenic mice**

To evaluate the cell-type specificity of Dec2FR activity in vivo, we generated transgenic mice bearing the Dec2FR-driven Luc gene. Various organs were excised and examined for Luc-specific activity (relative light units (RLU) per microgram of protein) (Fig. 5). Luc activity was highest in skin (from ear), with lower levels in lymphoid organs (spleen, lymph node, and thymus) known to contain relatively large numbers of DC. Nonlymphoid organs (e.g., adipose tissue, heart, and kidney) showed only background levels, although organs (lung, intestine, and testis) reported to harbor modest LC/DC densities showed similar Luc activity to lymphoid organ (Fig. 5) (28–30). The expression in skin becomes even more marked when Luc activity was normalized by DNA concentration. Because the ear is made up of skin (epidermis and dermis) and cartilage, we determined what tissue is a major source for the high activity. Luc activity was traced almost entirely to skin, with 80% in epidermis, 20% in dermis, and negligible activity in cartilage. Therefore, the highest Dec2FR activity appears linked to epidermal LC.

_Epidermal Luc activity is expressed exclusively by LC_  
Epidermal cells prepared from ear skin (pool of eight transgenic mice) expressed activity (8,023 RLU/10⁶ cells). Fractionation into
Activity was calculated as RLU/10⁴ cells and compared with that of epidermal LC (set at a value of 100%). Note that Luc activity for epidermal cells in offspring were assayed for the frequency of Ia⁺ and of fractions not bound to anti-Ia or anti-CD8 were collected and stained with magnetic beads revealed markedly high activity in the Ia⁺/H11001 fraction. The Luc activity (119,922 RLU/10⁴ cells), 15-fold higher than unfractionated epidermal cells (8,023 RLU/10⁴ cells). Selective isolation of DC, macrophages, T cells, and B cells was confirmed by marked reduction in frequency of Ia⁺ cells (from 4.8 to 0.05%) following depletion with anti-Ia treatment, but not with anti-CD8-coated beads (Fig. 6B). Therefore, Dec2FR activity is targeted to epidermal LC in the skin.

Epidermal LC is targeted for high expression of Luc among leukocytes

We next examined Luc expression levels in splenic DC and in non-DC leukocytes. Spleen cells prepared from the previous pool of transgenic mice showed 101 RLU/10⁴ cells, 80-fold lower than in epidermal cells, and this preparation was used to isolate splenic DC, T cells, and B cells. Splenic DC, purified using anti-CD11c Ab-magnetic beads, expressed 150-fold lower activity than epidermal LC (Fig. 7A). T and B cells, purified by flow cytometric sorting of CD3⁺ cells and CD45R/B220⁻ cells, respectively, both expressed mere background activity, documenting lack of Dec2FR influence in these cells. Finally, macrophages (Mac-1⁺) isolated from peritoneal cells elicited with thioglycolate displayed similar Luc activity as splenic DC. These findings lead us to conclude that epidermal LC are targeted selectively for high-level constitutive gene expression by Dec2FR in transgenic mice.

Discussion

Current consensus acknowledges DC to encompass different subsets that reside in various tissues, each subset bearing overlapping as well as distinct features. In this context, two possibilities exist with respect to the Dec2FR transcriptional regulatory unit. Dec2FR activity may be a feature common to all DC. Alternatively, it may be a distinguishing feature of only some DC subsets (like LC). We have shown Dec2FR activity (on a per-cell basis) to be expressed in epidermal Ia⁺ LC at differentially high levels compared with splenic DC. Dec2FR activity may be targeted to a certain subset of splenic DC which includes at least two distinct subpopulations with different phenotype and function (31, 32). In support of this possibility is our previous finding that dectin-2 molecule is expressed only by a subset (20%) of CD11c⁺ spleen DC (data not shown). Furthermore, we have not excluded the possibility that dermal DC express Dec2FR activity, although most certainly at very low levels if at all. Finally, LC reside not only in the epidermis but also in lung and mucosal tissues (e.g., intestine) (33). However, in our transgenic mice, Luc activity was markedly expressed in skin, with 10-fold lower activity per DNA base (Fig. 5) in lung and intestine, suggesting that Dec2FR transcriptional activity may also be targeted to a certain LC subset (epidermal but not lung or intestine).

Dec2FR contains cis-acting regulatory elements in the proximal region of mRNA initiation site, which are responsible for transcriptional activation by IFN-γ (IFN-stimulated response element),

![FIGURE 6. Epidermal Luc activity is expressed exclusively by LC. A. Epidermal cells prepared from ear skin of transgenic mice (a pool of eight offspring) were divided into three batches; one batch was not treated and the other batches were incubated with magnetic beads coated with rat anti-Ia or with anti-CD8 mAb (control). Unbound and bound fractions were separately collected and examined for cell number and Luc activity. These data were derived from two independent experiments. Ac. Luc activity is expressed as RLU/10⁴ cells. Ab. The anti-CD8-bound fraction was not counted. Ac. Values obtained in A were compared with those of epidermal cells (1.0). B. In parallel, small aliquots of unfractionated epidermal cells and of fractions not bound to anti-Ia or anti-CD8 were collected and stained with goat anti-rat IgG or rat anti-Ia Ab (for CD8-depleted). These samples were assayed for the frequency of Ia⁻ cells by flow cytometric analysis.

![FIGURE 7. Dec2FR activity is targeted to epidermal LC in transgenic mice. A. DC (CD11c⁺), B cells (B220⁻), and T cells (CD3⁺) were purified from spleen cells prepared from one pool of transgenic mice as was used in Expt. 1 of Fig. 6, using immunomagnetic beads or flow cytometric sorting. Peritoneal macrophages (90% Mac-1high) were isolated from peritoneal cells of a different pool of five transgenic mice stimulated with thioglycolate. Luc activity was calculated as RLU/10⁴ cells and compared with that of epidermal LC (set at a value of 100%). Note that Luc activity for epidermal cells in both pools of mice was similar (8,023 and 11,873 RLU/10⁴ cells). Selective isolation of DC, macrophages, T cells, and B cells was confirmed by flow cytometric analysis as performed in Fig. 6. B. Data are shown in a graphic form.](http://www.jimmunol.org/)
Our LC-targeted system will be used to develop new DNA vaccination strategies. DC (or APC)-targeting delivery of Ags induces rapid Ab responses following single step immunization (45) at 100 to 10,000-fold higher efficiency compared with nontargeted conventional protocols (46–47). Indeed, we have demonstrated recently that gene gun-mediated DNA delivery of the Dec2FR genetic element leads to efficient induction of immunity against a surrogate Ag (48).

In conclusion, Dec2FR-based LC-targeted gene expression represents a new opportunity to manipulate gene expression in LC, to better understand LC function, and to develop more effective ways of activating and regulating immune responses for preventing and treating diseases.

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References


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