analyze the presence of the splicing stimulation activity in various B cell lines at different developmental stages with the microinjection system described here as well as to investigate whether this activity in WEHI-231 nuclei also affects differential processing of other heavy chain genes.

We previously reported that μ mRNA transcribed from the Moloney murine sarcoma virus long terminal repeat promoter was processed exclusively to the secreted form in mouse fibroblast cell lines (*7*). Analyses of the expression of mutated mouse μ genes in various mouse cell lines suggested that splicing of the C4–M1 intron is positively regulated in mature cells (*7*). These results are consistent with our present observation using the *Xenopus* oocytes microinjection system and strengthen the conclusion that mature B cells contain one or more nuclear factors that specifically stimulate splicing of the C4–M1 intron.

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26. We thank L. Herzenberg and M. Koshland for cell lines, S. McKnight for the plasmid containing the SV40/HSV-TK hybrid promoter, and J. Tso, D. Guinta, and C. Queen for valuable discussions and critical reading of the manuscript. Supported by NIH grant AI21298 to L.J.K. N.T. was a postdoctoral fellow of the Damon Runyon–Walter Winchell Cancer Fund (DRG-857). During part of this work, L.J.K. was an American Cancer Society Junior Faculty Research Awardee.

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The CD14 Monocyte Differentiation Antigen Maps to a Region Encoding Growth Factors and Receptors

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CD14 is a myelomonocytic differentiation antigen expressed by monocytes, macrophages, and activated granulocytes and is detectable with the monoclonal antibodies MO2, MY4, and LeuM3. Analyses of complementary DNA and genomic clones of CD14 show that it has a novel structure and that it maps to chromosome 5 within a region containing other genes encoding growth factors and receptors; it may therefore represent a new receptor important for myeloid differentiation. In addition, the *CD14* gene is included in the "critical" region that is frequently deleted in certain myeloid leukemias.

DIFFERENTIATION OF MYELOMONOCYTIC cells from pluripotent stem cells to mature, functioning monocytes/macrophages and granulocytes is accompanied by a variety of changes including the expression of new cell surface antigens (*1*). One such antigen, CD14, recognized by a number of monoclonal antibodies (mAbs) including MO2, MY4, and LeuM3 (*2, 3*), is a 55-kD glycoprotein expressed by monocytes, macrophages (*4, 5*), and activated granulocytes (*5, 6*). Although no biological function has yet been ascribed to this molecule, its restricted expression on mature cells suggests an important effector function. A complementary DNA (cDNA) clone encoding CD14 was isolated by means of a novel method that includes cell surface screening with monoclonal antibodies of COS 7 cells transfected with expressible cDNA clones. In addition, the *CD14* gene was isolated, characterized, and found to be located on chromosome 5 in a region containing a number of growth factor and receptor genes.

A cDNA library was constructed in the Okayama/Berg eukaryotic expression vector, pCD (*7*), with messenger RNA (mRNA) isolated from M4-AML (myelomonocytic) cells, which express high levels of CD14 (*2*). A cDNA clone encoding CD14 was isolated

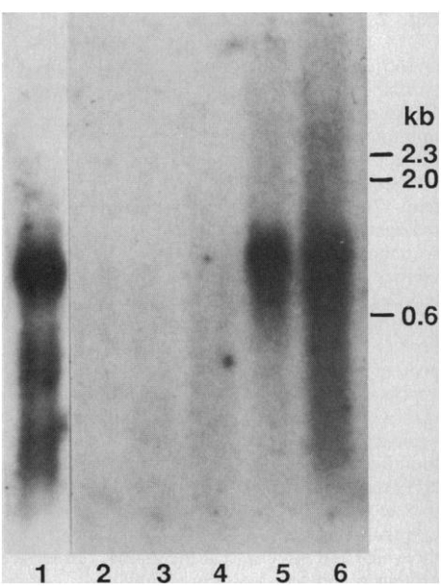

Fig. 1. Northern blot analysis of pCD-CD14 transcripts in hematopoietic cells. Total RNA was isolated from cells at various stages of differentiation on cesium chloride gradients (*16*) and polyadenylated RNA was isolated by oligo(dT) affinity chromatography (*17*). Total RNA (20 μg) (lanes 4 to 6) or polyadenylated RNA (1 μg) (lanes 1 to 3) was separated by electrophoresis in denaturing agarose gels, transferred to nitrocellulose, and hybridized with ^{32}P-labeled nick-translated CD14 cDNA (*18*). The migration of λ DNA digested with Hind III is noted. (Lane 1) EF(M4-AML); (lane 2) GM3103 (B cell line); (lane 3) HSB2 (T cell line); (lane 4) lymphocytes; (lane 5) monocytes; and (lane 6) granulocytes.

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by immunofluorescent analysis of transfected COS 7 cells, with a mAb to CD14 as described (*8*). To confirm that this clone, pCD-CD14, encodes authentic CD14 molecules, we first compared by SDS–polyacrylamide gel electrophoresis (PAGE) CD14 immunoprecipitates from pCD-CD14–transfected COS 7 cells and from M4-AML cells expressing endogenous CD14. The molecules precipitated from both sources were nearly identical in size (*2*). We next used Northern blot analysis to determine whether pCD-CD14 hybridized with mRNA expressed at the appropriate stages of differentiation. RNA isolated from cells representing different stages of myeloid differentiation as well as different cell lineages was analyzed for its ability to bind the pCD-CD14 probe. This probe was found to hybridize to a single mRNA species that showed an expression profile identical to CD14; it was present in monocytes, granulocytes, and M4-AML cells (Fig. 1), but not in less mature myeloid cells represented by the leukemic cell lines K562 (undifferentiated), U937 (monoblast-like), HL60 (promyelocyte-like), or M2-AML (myeloblastic with maturation) cells (data not shown) or lymphocytes. Finally, the predicted protein sequence of the pCD-CD14 clone corresponded to the partial protein sequence of CD14 determined by microsequence analysis (Fig. 2).

The pCD-CD14 cDNA clone was found to consist of 1367 nucleotides with a polyadenylate tail at the 3′ end. Its sequence is available upon request and will be submitted to GenBank. An initiation codon was identified at position 105, followed by an open reading frame consisting of 1125 nucleotides and flanked by 104 nucleotides of 5′ untranslated sequence and 126 nucleotides of 3′ untranslated sequence. Comparison with the partial protein sequence determined by microsequence analysis confirms the identity of this clone as encoding CD14 (Fig. 2) and indicates the presence of a signal peptide of 19 amino acids.

The CD14 protein sequence contains several features characteristic of cell surface glycoproteins. These include a stretch of 17

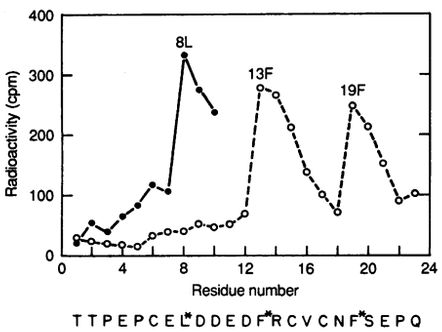

Fig. 2. Partial NH_2-terminal sequence of the CD14 cell surface protein and comparison to amino acid sequence deduced from cDNA. The partial NH_2-terminal amino acid sequence of CD14 molecules purified from M4-AML cells by immunoprecipitation and SDS-PAGE was determined by radioactive sequence analysis of [^{3}H]leucine- and [^{3}H]phenylalanine-labeled proteins (*19*). Counts above background were plotted on a linear graph. The [^{3}H]leucine sample was sequenced for 10 cycles and the [^{3}H]phenylalanine sample was sequenced for 23 cycles. A leucine residue (solid line) was detected at amino acid position 8 and phenylalanine residues (broken line) were detected at positions 13 and 19. The protein sequence [as indicated by the one-letter amino acid code (*20*)] of CD14 deduced from the cDNA clone is shown under the graph. As can be seen, leucine and phenylalanine residues (*) are found at identically spaced positions near the NH_2-terminus of the pCD-CD14–predicted protein sequence. These results establish the NH_2-terminal amino acid of the mature protein as threonine and indicate that the mature cell surface protein is composed of 356 amino acids.

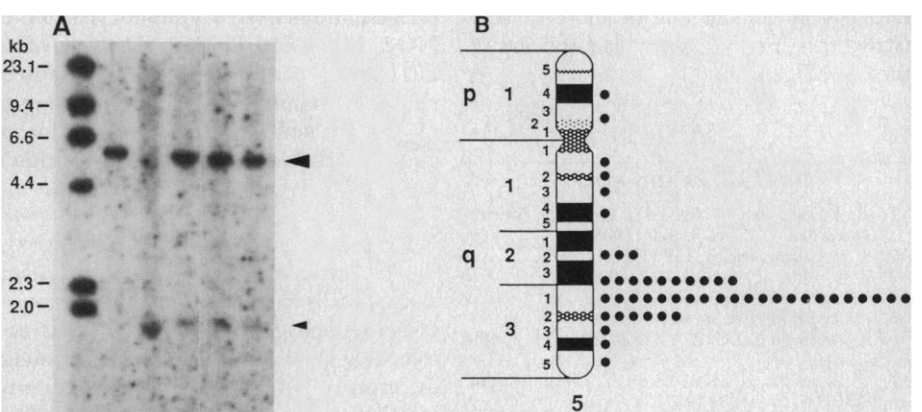

Fig. 3. (**A**) Southern blot analysis of Eco RI digests of DNA isolated from human, mouse, and selected human × mouse hybrids. Restriction endonuclease–digested genomic DNA was separated on 0.7% agarose gels, transferred to nitrocellulose, and hybridized with ^{32}P-labeled nick-translated CD14 cDNA (*16*). Filters were washed in 0.3× standard saline citrate with 0.1% SDS at 65°C. Sizes are in kilobases. (Lane 1) Hind III–digested λ DNA molecular weight standard; (lane 2) human DNA; (lane 3) mouse DNA; (lane 4) hybrid NSK-1; (lane 5) hybrid NSK-1s; and (lane 6) hybrid ANK3.1. Large and small arrowheads indicate, respectively, the human and mouse CD14 hybridizing sequences. (**B**) Distribution of labeled sites on chromosome 5 in normal human metaphase cells that were hybridized with the *CD14* probe. Of 100 metaphase cells examined, 36 (36%) were labeled on region q2 or q3 of one or both chromosome 5 homologs. Of 182 labeled sites observed, 50 (27.5%) were located on this chromosome. These sites were clustered at bands q22 to q32, and this cluster represented 22.5% (41/182) of all labeled sites (cumulative probability for the Poisson distribution is <0.0005). The largest number of grains was located at 5q23 to q31. All hybridizations were repeated three times and gave similar results in each experiment. Human metaphase cells were prepared from phytohemagglutinin-stimulated peripheral blood lymphocytes. Radiolabeled *CD14* probes were prepared by nick translation of the entire plasmid with all four ^{3}H-labeled deoxynucleoside triphosphates to a specific activity of 1.0×10^8 dpm/μg. In situ hybridization was performed as previously described (*10*). Metaphase cells were hybridized at 4.0 and 8.0 ng of probe per milliliter of hybridization mixture. Autoradiographs were exposed for 11 days.

Table 1. Discordancy analysis for the chromosomal assignment of human *CD14*. Twenty-one mouse-human somatic cell hybrids were examined for their human chromosome content by karyotype analysis and by testing for human isoenzymes, cell surface antigens, and DNA markers (*9*). Presence or absence of human *CD14* was determined by Southern blot analysis of Eco RI–digested hybrid cell DNA with a ^{32}P-labeled nick-translated CD14 cDNA probe. Numbers listed refer to hybrids showing discordance for the presence of specific human chromosomes and human *CD14*; deleted or rearranged copies of human chromosomes were not evaluated.

Analysis	Human chromosome																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
No. discordant	6	7	7	9	0	11	5	9	5	7	5	10	5	11	12	8	8	6	8	14	13	5	10	7
No. concordant	3	13	12	10	21	9	15	12	15	10	3	10	16	9	7	12	10	15	8	6	6	15	11	14

Table 2. In situ hybridization of the human pCD-CD14 probe to leukemia metaphase cells from five patients with a del(5q).

Patient	Hematologic disease*	Deletion	Number of cells analyzed/ number of labeled sites	Number of labeled sites (%)			
				Normal chromosome 5		5q− chromosome	
				Total	Bands q22–q32	Total	Bands q13–q35
1	RA	del(5)(q15q33.3)	73/167	30 (18.0%)†	20 (12.0%)	6 (3.6%)	3 (1.8%)
2	RA	del(5)(q13q33.3)	40/88	20 (22.7%)†	12 (13.6%)	2 (2.3%)	0
3	RA	del(5)(q22q33.3)	90/110	15 (13.6%)†	14 (12.7%)	4 (3.6%)	2 (1.8%)
4	ANLL	del(5)(q15q34)	100/120	21 (17.5%)†	15 (12.5%)	1 (0.8%)	0
5	t-ANLL	del(5)(q14q33.3)	100/175	26 (14.8%)†	19 (11.0%)	5 (2.9%)	1 (0.6%)

*RA, refractory anemia 5q− syndrome; ANLL, acute nonlymphocytic leukemia; t-ANLL, therapy-related acute nonlymphocytic leukemia. †Cumulative probability for the Poisson distribution is <0.0005. The mean was estimated from the number of labeled sites on all chromosomes except the normal 5 and del(5q) chromosomes.

hydrophobic and neutral amino acids at the COOH-terminus, which could serve as a membrane anchor, and five potential sites for *N*-linked glycosylation. Comparison of the CD14 nucleotide and predicted protein sequences to all sequences in the Bionet data bank shows no significant homologies.

The *CD14* gene was isolated from a size-selected (6-kb average) Eco RI genomic library constructed in the λ vector gtWes. DNA sequence analysis indicates that it contains a single intron of 88 base pairs immediately after the ATG translational start site. Southern blot analysis of DNA digested with several different restriction enzymes and probed with CD14 cDNA gave single bands, suggesting that CD14 is encoded by a single gene (data not shown).

The chromosomal location of the human CD14 gene (*CD14*) was determined by analysis of DNA from mouse-human somatic cell hybrids. Of 21 hybrid clones, 6 were positive for the 5.5-kb Eco RI gene fragment (examples shown in Fig. 3A). These six hybrids were found by karyotype analysis and testing for human isoenzymes, cell surface antigens, and DNA markers (*9*) to contain human chromosome 5 but to have no other human chromosome in common (Table 1). Furthermore, none of the 15 hybrids that were negative for human *CD14* contained a complete copy of human chromosome 5. In situ chromosomal hybridization (*10*) of the ^{3}H-labeled pCD-CD14 probe to normal human metaphase cells resulted in specific labeling only of chromosome 5 (Fig. 3B). The labeled sites were clustered at 5q22–q32; the largest cluster of grains was located at 5q23–q31.

The localization of the gene encoding CD14 (a differentiation antigen specifically expressed by myeloid cells) to 5q23–q31 is particularly interesting in light of the nonrandom chromosomal deletions in human myeloid disorders that involve this chromosome region. Loss of a whole chromosome 5 or loss of a part (deletion) of the long arm of this chromosome [del(5q)] is frequently observed in the malignant cells of patients with a myelodysplastic syndrome [(MDS) 5 to 10%], therapy-related acute nonlymphocytic leukemia [(t-ANLL) 50%], or acute nonlymphocytic leukemia de novo [(ANLL) 10%] (*11, 12*). Although there is variability in the breakpoints of these interstitial deletions, cytogenetic analysis has revealed that one segment of chromosome 5 is deleted in all patients who have this cytogenetic abnormality (*11*); this segment, called the critical region, consists of bands 5q23–q31. The identification of such a region suggests that loss of a critical DNA sequence leading to hemizygosity (or homozygosity) of a recessive allele may play an important role in the pathogenesis of these disorders. Such a mechanism has been substantiated recently for retinoblastoma (*13*). To determine the relation of the *CD14* gene to the critical region of chromosome 5, we hybridized the pCD-CD14 probe to metaphase cells obtained from bone marrow aspirates of five patients who had an MDS (5q− syndrome), ANLL de novo, or t-ANLL characterized by a del(5q). This resulted in specific labeling of the normal chromosome 5 homologs, but not of the rearranged homologs (Table 2), indicating that the *CD14* locus was deleted as a result of an interstitial deletion of 5q.

Our data indicate that the *CD14* gene is located in a region of chromosome 5 containing a cluster of genes that encode several myeloid-specific growth factors [interleukin-3, granulocyte-macrophage colony-stimulating factor, and macrophage colony-stimulating factor (CSF-1)] or growth factor receptors [FMS, receptor for CSF-1 (*14*)], as well as other growth factor and receptor genes [platelet-derived growth factor receptor, β2-adrenergic receptor, and endothelial cell growth factor (*15*)]. The mapping of the *CD14* gene to this region of chromosome 5, its expression preferentially by mature myeloid cells, and its deletion in the malignant cells of patients having myeloid leukemias and a del(5q) suggest that the CD14 antigen may also serve as some type of receptor and that a role for this gene in the pathogenesis of myeloid disorders should be considered.

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20. Abbreviations for the amino acid residues are: C, Cys; D, Asp; E, Glu; F, Phe; L, Leu; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; and V, Val.
21. Supported by NIH grant R01-AI23859 (S.M.G.), U.S. Department of Energy contract No. DE-FG02-86ER60408, USPHS grant CA 16910 (J. D. Rowley), and the University of Chicago Cancer Research Foundation (M.M.LeB). M.M.LeB. is a Special Fellow of the Leukemia Society of America. We thank P. Gregersen for the genomic library, C. Hurley for the amino acid sequence analysis, S. Seremetis for the AML cells, R. J. Winchester for the MoS39 mAb, R. Espinosa III for technical assistance, Y.-T. Chen for valuable contributions, A. Rupel for manuscript preparation, and J. Silver for many helpful discussions.

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Prevention of Type I Diabetes in Nonobese Diabetic Mice by Virus Infection

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The nonobese diabetic (NOD) mouse is an animal model of type I diabetes and develops a characteristic autoimmune lesion in the islets of Langerhans with lymphocytic infiltration and destruction of pancreatic β cells. The result is hypoinsulinemia, hyperglycemia, ketoacidosis, and death. Diabetes usually begins by the sixth month of age but can occur earlier when young NOD mice are infused with lymphocytes from older NOD donors. When newborn or adult NOD mice were infected with a lymphotropic virus they did not become diabetic. The interaction between viruses and lymphocytes is pivotal in aborting diabetes, as established by experiments in which lymphocytes from virus-infected donors failed to transfer diabetes. In contrast, lymphocytes from age- and sex-matched uninfected donors caused disease. Therefore, viruses and, presumably, their products can be developed to be beneficial and may have potential as a component for treatment of human diseases. Further, these results point to the utility of viruses as probes for dissecting the pathogenesis of a nonviral disease.

VIRUSES ARE KNOWN BY THE CELLS they injure or diseases they cause. According to P. and J. Medawar (*1*), "viruses make themselves known only by causing disease or other pathological changes; the existence of benign viruses having no ill effects remains conjectural. No virus is known to do good. It has been well said that a virus is a piece of bad news wrapped up in protein."

Certain viruses infect and replicate in lymphocytes, thereby disordering their function and causing immune suppression or heightened autoimmune responses (*2, 3*). Perhaps viruses could be directed to induce selective immune suppression in an autoimmune disorder, with the potential to prevent the autoimmune response and concomitant disease. This hypothesis was tested in a study of the nonobese diabetic (NOD) mouse, which spontaneously develops insulin-dependent diabetes mellitus (IDDM), usually beginning by 6 months of age, with an incidence of nearly 100% by the 9th to 12th month. The diabetes is autoimmune and involves lymphocytic infiltration around and into the islets of Langerhans with pancreatic β cell destruction. The result is hypoinsulinemia, hyperglycemia, ketoacidosis, and death, as in human IDDM (*4–6*).

Lymphocytic choriomeningitis virus (LCMV) is an ambisense bisegmented RNA virus that is a natural pathogen of mice (*7–9*). In most murine strains, injection at birth (immunoincompetent host) or in adulthood (immunocompetent host) with a lymphotropic variant of LCMV results in infection of lymphocytes, primarily of the T-helper subset (Thy 1.2$^+$, L3T4$^+$, Lyt-2$^-$) (*10, 11*). The outcome is abrogation of virus-specific H-2–restricted cytotoxic T lymphocyte function, failure to clear virus, and viral persistence throughout the animal's lifespan (*9, 12*). LCMV infection initiated in NOD mice at birth or in adulthood abrogated the expected incidence of IDDM and normalized blood glucose and pancreatic insulin levels. Furthermore, adoptive transfer of lymphocytes from LCMV-infected NOD mice into uninfected NOD recipients prevented or minimized the autoimmune lesions within islets; in contrast, NOD mice littermates that received lymphocytes from uninfected mice had clear-cut IDDM.

The NOD colony was established from breeder mice by brother-sister matings. The incidence of IDDM (defined as blood glucose greater than 300 mg/dl) in twenty 9-month-old mice was 95%, with the mean ± SEM blood glucose of 454 ± 37 mg/dl.

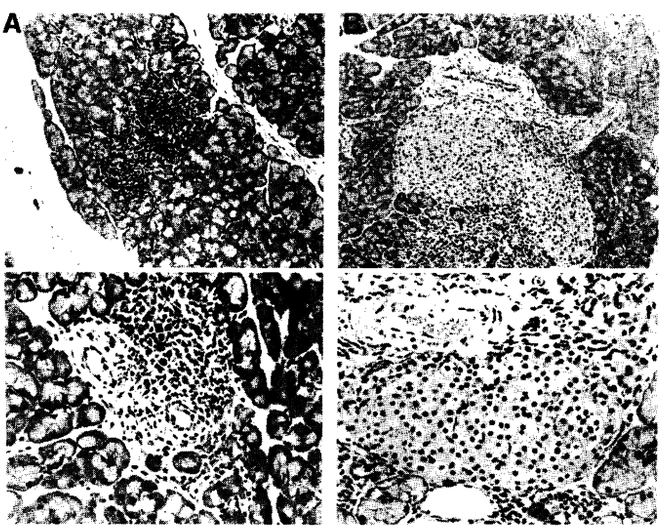

Fig. 1. Histopathology of pancreatic islets of NOD mice 30 days after exposure to radiation (850 rads) and subsequent adoptive transfer of lymphocytes and bone marrow cells from (**A**) an uninfected NOD mouse and (**B**) a NOD mouse infected at birth with LCMV. Massive islet cell destruction and lymphocytic infiltration can be observed in two separate mice receiving lymphocytes from the uninfected donor (A) as contrasted to two mice receiving lymphocytes from virus-infected donors and failing to develop IDDM. Magnifications in (A) and (B): top, ×250; bottom, ×450. These results are representative of individual mice in the experimental groups recorded in Table 2. See Table 2 for experimental details.

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