JOURNAL OF VIROLOGY, June 1997, p. 4857–4861 Vol. 71, No. 6
0022-538X/97/$04.00+0

A Homolog of Interleukin-10 Is Encoded by the Poxvirus Orf Virus

STEPHEN B. FLEMING,[1]* CATHERINE A. McCAUGHAN,[1] ARNA E. ANDREWS,[2] ANDREW D. NASH,[2] AND ANDREW A. MERCER[1]

Health Research Council Virus Research Unit and University of Otago Centre for Gene Research, University of Otago, Dunedin, New Zealand,[1] and Centre for Animal Biotechnology, School of Veterinary Science, University of Melbourne, Parkville 3052, Australia[2]

Received 27 December 1996/Accepted 11 March 1997

A gene encoding a polypeptide with homology to interleukin-10 (IL-10) has been discovered in the genome of orf virus (OV) strain NZ2, a parapoxvirus that infects sheep, goats, and humans. The predicted polypeptide sequence shows high levels of amino acid identity to IL-10 of sheep (80%), cattle (75%), humans (67%), and mice (64%), as well as IL-10-like proteins of Epstein-Barr virus (63%) and equine herpesvirus (67%). The C-terminal region, comprising two-thirds of the OV protein, is identical to ovine IL-10, which suggests that this gene has been captured from its host sheep during the evolution of OV. The IL-10-like gene is transcribed early. Conditioned medium from COS cells transfected with a eukaryotic expression vector containing the OV IL-10-like gene showed the same biological activity as ovine IL-10 in a murine thymocyte proliferation assay. OV IL-10 is likely to be important in immune evasion by OV, since IL-10 is a multifunctional cytokine that has inhibitory effects on nonspecific immunity and Th1 effector function.

Orf virus (OV) is the type species of the parapoxvirus genus and infects sheep, goats, and humans (24). OV causes an acute contagious skin disease that lasts for 2 to 4 weeks, after which time the lesion resolves. The virus replicates in regenerating epidermal keratinocytes (17) and has no systemic phase. Animals can be infected repeatedly with OV, although the size of the lesion and time to resolution diminishes with each exposure (10, 17, 24). This phenomenon has raised questions about the mechanisms underlying this apparent escape from host immunity.

Many poxviruses encode virulence factors that subvert the defense mechanisms of their hosts (reviewed in references 1, 23, and 29). These factors either directly inhibit or modify the early stages of the host response during viral replication. In some cases these factors are homologous with host immune proteins, suggesting that poxviruses have captured host genes during the process of evolution. Some poxviruses encode soluble factors that are secreted from virus-infected cells and mimic host cytokine receptors. Examples of such soluble receptors include poxvirus proteins with sequence similarity for receptors of tumor necrosis factor, interleukin-1 (IL-1), gamma interferon, and alpha/beta interferon. In addition, poxviruses encode serpins, factors that inhibit signal transmission pathways involved in apoptosis, and molecules that are homologous to host growth factors.

We are interested in identifying genes in OV that may be involved in pathogenesis and immune modulation. DNA sequence analysis of various parts of the OV genome has revealed that the genetic structure of OV resembles that of vaccinia virus, the prototypal member of the orthopoxvirus genus (9, 19). The vaccinia virus genome is approximately 190 kb, and the essential genes of that virus appear to be conserved in the 139-kb genome of OV. We have deduced that the difference in size of the genomes can largely be attributed to differences within the termini. In vaccinia virus the terminal regions contain genes involved in pathogenesis, virulence, and host range and are often nonessential for the replication of the virus in cell culture (5, 31). We have sequenced 12 kb of genomic DNA at the left end of OV and have shown that most of the genes within this region have homologs in vaccinia virus but are of unknown function (19). At the right end of the genome we have found homologs of the vaccinia virus genes F9 and F10 (19) and a homolog of vascular endothelial growth factor (15).

In this study we sequenced both strands of a 6.0-kb DNA fragment derived from the right terminus of the OV NZ2 genome. The NZ2 strain of OV was isolated in New Zealand (25) and propogated in bovine testis cells as described previously (4). The fragment sequenced here spans a region from OV 133.8R (vaccinia virus F10 homolog) to the *Kpn*I-E/K junction (19). DNA subclones of the 6.0-kb fragment were prepared with a nested deletion kit (Pharmacia P-L Biochemicals), and double-stranded DNA templates were prepared and sequenced by procedures recommended by Applied Biosystems Inc. (ABI). Reagents used for sequencing were supplied by ABI, and the products of the sequencing reactions were analyzed with an ABI model 373A sequencing system.

The DNA sequence derived from the 6.0-kb fragment was translated in all six reading frames with the program Blast (2), and the translated sequences were compared with protein sequences in the PIR database (release 46.0) held by GenBank. This analysis revealed an open reading frame with a high degree of identity to mammalian IL-10 (6, 12, 20, 32) and IL-10-like genes of members of the herpesvirus family, Epstein-Barr virus (EBV) (3, 20), and equine herpesvirus 2 (EHV2) (27). The putative gene was termed OV NZ2-IL-10.

The initiation codon of the OV NZ2-IL-10 gene is located 10 kb from the right end of the genome, and the gene is transcribed in a rightward direction. The DNA sequence and the translated sequence of the coding region are shown in Fig. 1. The remainder of the sequence of the 6.0-kb fragment will be published elsewhere. A comparison of the predicted amino acid sequence of OV NZ2-IL-10 with those of other IL-10 genes gave optimized scores which ranged from 745 to 594. Ovine IL-10 gave the highest score of 745, with 96% identity over 148 amino acids (aa). The OV NZ2-IL-10 gene is 561 nucleotides (nt) and, unlike cellular IL-10 genes but in keeping

* Corresponding author. Mailing address: HRC Virus Research Unit, P.O. Box 56, Dunedin, New Zealand. Phone: 64 (03) 4797-727. Fax: 64 (03) 479-7744. E-mail: steve@sanger.otago.ac.nz.

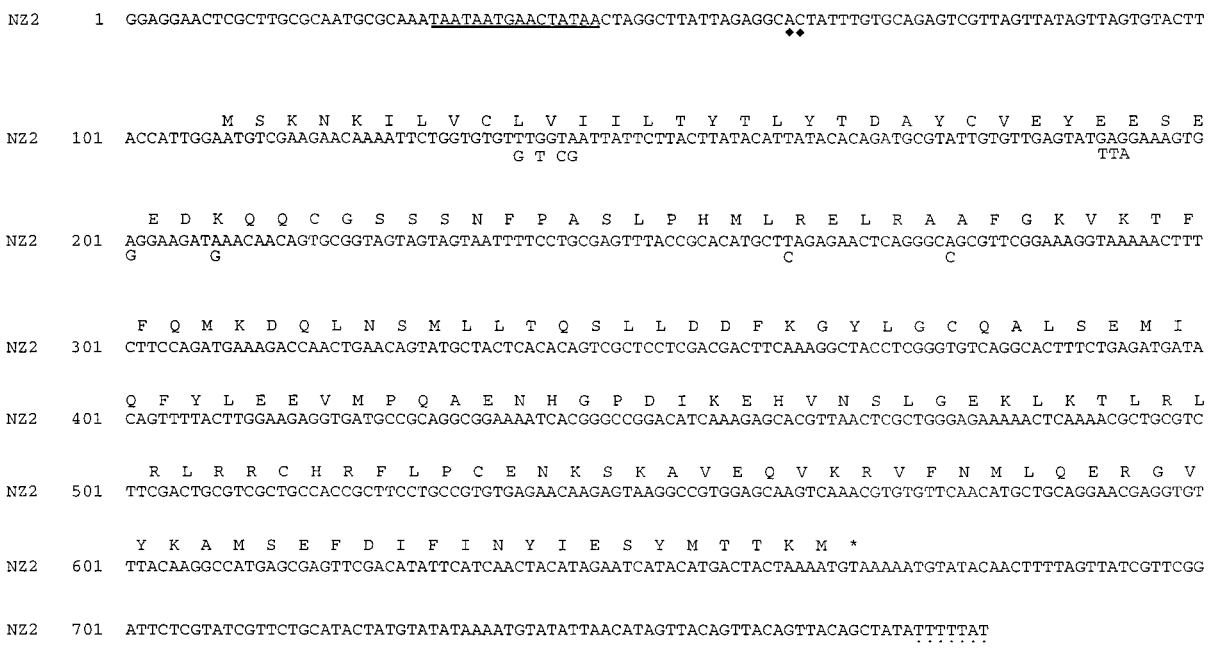

FIG. 1. Nucleotide sequence of the OV NZ2 IL-10-like gene. The order of amino acids, represented in one-letter code, was deduced from the nucleotide sequence. Differences in nucleotide sequence in OV NZ7-IL-10 are shown below the OV NZ2 sequence. Nucleotides in OV NZ2 which are not present in OV NZ7 are indicated by diamonds. The putative early promoter sequence is underlined, and the early transcription termination signal sequence is indicated by dots.

with other poxvirus genes, does not contain introns. The length of the predicted polypeptide encoded by OV NZ2-IL-10 is 186 aa with a molecular mass of 21.742 kDa. This makes the OV NZ2-IL-10 polypeptide slightly larger than its cellular and viral counterparts, which range in size from 170 aa for BCRF1 (EBV) (3) to 179 aa for the EHV2 IL-10-like gene product (26, 27).

We were interested in finding whether an IL-10-like gene is found in other strains of OV and to establish if the IL-10-like gene found in OV NZ2 is likely to be typical for this species. The IL-10-like gene was PCR amplified in OV strain NZ7 by methods described previously (14). *Taq* polymerase (Boehringer Mannheim) was used in all PCR amplifications. The primers used for PCR amplification were 5′-ATGTCGAAGA ACAAAATTCT and 5′-TTACATTTTAGTAGTCATGT and were based on the 5′ and 3′ ends of the OV NZ2-IL-10 gene, respectively. The PCR amplification assay was carried out using the cloned restriction fragment *Kpn*I-E derived from OV NZ7 (15), which maps to the same region of the genome as *Kpn*I-E in OV NZ-2. The PCR amplification assay gave a major product of approximately 550 nt as determined by agarose gel electrophoresis. This product was purified and partly sequenced to establish that it encoded an IL-10-like sequence. We established the true sequence by sequencing both strands of the IL-10-like gene within the *Kpn*I-E (NZ-7) fragment with specific primers. This gene was designated OV NZ7-IL-10.

We deduced from this analysis that the IL-10 gene homologs in OV strains NZ-2 and NZ-7 are identical in length with few differences at the nucleotide level (Fig. 1). Differences in the translated sequences were found near the N terminus only (see Fig. 3). Restriction endonuclease analysis and Southern blotting showed that the OV IL-10 gene homologs map to the same position on the genome (data not shown).

The sequences flanking each of the OV IL-10 gene homologs are very similar to each other, and these sequences are typical of poxvirus early transcriptional regulatory sequences. An A+T-rich early promoter-like sequence (7, 8, 22) is found 62 nt upstream from the initiation codon, while an early transcriptional termination sequence (22), TTTTTAT, is found 101 nt downstream from the stop codon (Fig. 1). The upstream and downstream sequences of the OV IL-10 gene homologs are highly conserved. The putative early promoter sequence is identical in each strain, and the downstream sequences are conserved (Fig. 1).

Northern blot analysis was conducted with early RNA derived from bovine testis cells infected with either OV strain NZ2 or OV strain NZ7. Cells were infected with virus in the presence of cycloheximide in order to block protein synthesis and subsequent transcription of potential intermediate and late genes. Extraction of RNA from virus-infected cells and Northern blotting analysis was carried out by procedures described previously (7). Hybridization of early RNA with a double-stranded DNA (dsDNA) probe specific for OV NZ2-IL-10 (nt 110 to 670 [Fig. 1]) revealed two major transcripts of 0.8 and 2.3 kb (Fig. 2). Quantitative differences were found in the relative levels of these transcripts in the two strains of OV. The 2.3-kb transcript was produced in greater abundance than the 0.8-kb transcript in the case of RNA derived from cells infected with OV NZ2, whereas the reverse was found with RNA derived from cells infected with OV NZ7. We deduced from the sequence data that mRNAs of approximately 760 nt would be expected to be transcribed from the IL-10-like gene in each strain of OV.

We suspected that the 2.3-kb transcript originated from an early gene upstream of the IL-10-like gene in each strain and terminated downstream of the IL-10-like gene. To show whether this was the case, a dsDNA probe which spanned a region from the 5′ end of the OV NZ2-IL-10 putative promoter (nt 4 [Fig. 1]) to a site 500 bp upstream of the OV NZ2 IL-10-like gene was hybridized with early RNA. The results showed that this probe hybridized with a transcript of 2.3 kb in each case (Fig. 2). In addition, the 2.3-kb transcript was shown

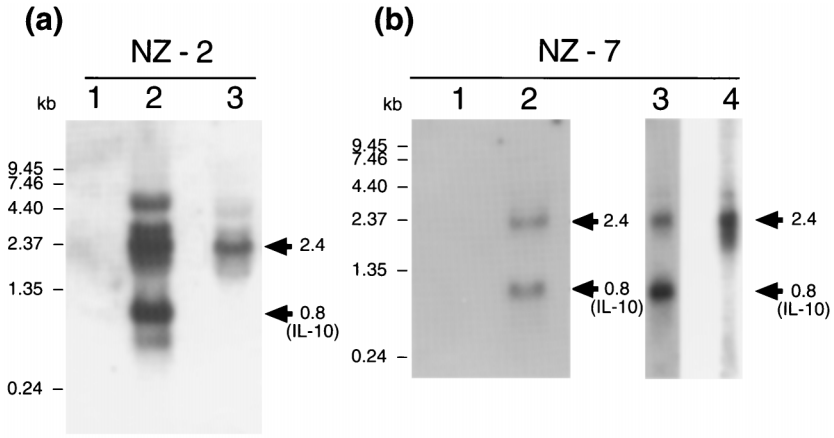

FIG. 2. Northern analysis of the IL-10 gene. Total RNA was isolated from bovine testis cells infected with either OV NZ2 or OV NZ7. Early RNA was isolated 6 h postinfection from cells infected in the presence of cycloheximide. RNA was separated by electrophoresis in an agarose-formaldehyde gel and transferred to a nitrocellulose membrane. All membranes were hybridized with ^{32}P-labelled dsDNA probes. The IL-10 probe spanned the coding sequence of the IL-10 gene. The upstream probe spanned a region from the 5′ end of the putative IL-10 promoter (nt 4 [Fig. 1]) to a site 500 bp upstream of the IL-10 gene. (a) NZ-2. Lane 1, mock-infected RNA hybridized with IL-10 probe; lane 2, early RNA hybridized with IL-10 probe; lane 3, early RNA hybridized with upstream probe. (b) NZ-7. Lane 1, mock-infected RNA hybridized with IL-10 probe; lanes 2 and 3, early RNA hybridized with IL-10 probe; lane 4, early RNA hybridized with upstream probe. The blot shown in lanes 3 and 4 was prepared from a gel different from that shown in lanes 1 and 2. The positions of the RNA size markers are shown to the left.

to be transcribed from the same strand as the 0.8-kb transcript by hybridization with a single-stranded probe which spanned a region 47 nt to the left of nt 1 (Fig. 1) to nt 246 (Fig. 1) (data not shown). Finally, to explain the transcriptional read-through observed for the 2.3-kb early transcripts, we sequenced 1,500 nt of the genome upstream of OV NZ2-IL-10 and 500 nt upstream of OV NZ7-IL-10. This analysis did not reveal any early transcription termination motifs (data not shown).

An alignment of the predicted amino acid sequences of the products of OV NZ2-IL-10, OV NZ7-IL-10, the ovine IL-10 gene (6), the bovine IL-10 gene (12), the human IL-10 gene (32), the mouse IL-10 gene (20), the EBV IL-10-like gene (BCRF1) (20), and the EHV2 IL-10-like gene (26) is shown in Fig. 3. The alignment was conducted with the program Clustal (13). Examination of this alignment showed that the translated sequences of the OV IL-10-like genes are very similar to sequences of other IL-10s.

The homologies of the predicted polypeptide sequences of OV NZ2-IL-10 and OV NZ7-IL-10 with mammalian and viral IL-10-like proteins are as follows: ovine, 80 and 79%, respectively; bovine, 75 and 74%; human, 67 and 67%; mouse, 64 and 63%; EBV, 63 and 62%; and EHV2, 67 and 66%. The identity of the OV IL-10-like genes is highest over the final two-thirds of the protein. This region of the gene is highly conserved across all mammalian species of IL-10 and herpesvirus IL-10-like genes. More remarkable is that the OV IL-10 homologs are 98.6% identical with ovine IL-10 from aa 44 to the carboxy terminus. The relatedness of OV IL-10 to ovine IL-10 is less apparent at the DNA level (67% identity). This reflects differences in codon usage and the higher G+C content of OV genes in general.

Less homology is seen when the N-terminal ends of the OV IL-10 polypeptides (aa 1 to 42) are compared with corresponding regions of mammalian and other viral IL-10s. Part of this region is likely to form the secretory signal sequence of OV IL-10. A domain which falls within the secretory signal sequences of mammalian and herpesvirus IL-10s and has the consensus sequence ALLCCLVLLT/A is partly conserved in the OV IL-10 homologs. In addition, a hydrophilicity plot (Kyte-Doolittle) revealed a strongly hydrophobic region of 18 aa at the N terminus of the OV IL-10 polypeptides. A secretory signal sequence of approximately 18 aa fits closely with the lengths of the putative secretory sequences of mammalian and EHV IL-10s, which are between 18 and 19 aa (6, 12, 16, 27, 32).

In order to determine if OV NZ2-IL-10 displays IL-10-like activity, the coding region of the IL-10 gene was PCR amplified from pVU89 and the product was cloned into the *Eco*RI and *Xba*I sites of eukaryotic expression vector FIII (16), containing the simian virus 40 origin of replication, enhancer, and polyadenylation signals and the human metallothionein promoter, $hMTII_A$. IL-10 was expressed transiently in COS cells which were transfected with recombinant plasmid FIII by the DEAE-dextran method (28). The resulting supernatants were harvested after 72 h and assayed for IL-10-like activity in a murine thymocyte proliferation assay containing recombinant human IL-2. Three- to eight-week-old female BALB/c mice were used as the source of thymocytes. Mice were sacrificed, and thymic lobes were excised. Single-cell suspensions were prepared by gentle teasing of the lobes through a sterile fine stainless steel mesh into medium. Assays were carried out in 96-well flat-bottom sterile microtiter plates containing appropriately diluted test samples and controls, recombinant human IL-2 (250 U/ml), and 3×10^5 thymocytes in a final volume of 200 μl per well. The cells were pulsed with 1 μCi of [^{3}H]thymidine during the final 18 to 24 h of a 120-h culture period. Thymocytes were harvested, and [^{3}H]thymidine uptake, as an indicator of cellular proliferation, was determined by liquid scintillation counting. The results showed that the plasmid containing the OV IL-10-like gene expressed a protein with a biological activity which, in this assay, is indistinguishable from ovine IL-10 (Fig. 4). The detection of the activity in the conditioned medium suggests that the OV protein is secreted. We also found that a clone in which a single point mutation had occurred during the PCR amplification of the OV IL-10 gene did not affect its activity in this assay. The mutation involved the substitution of thymine for adenine at nt 384 (Fig. 1) and would result in an amino acid change from lysine to asparagine.

IL-10 is a multifunctional cytokine that has suppressive effects on inflammation, antiviral responses, and T-helper type 1 (Th1) effector function (reviewed in reference 21). These activities of IL-10 suggest that OV IL-10 can subvert both innate immunity and specific cellular immune responses in the in-

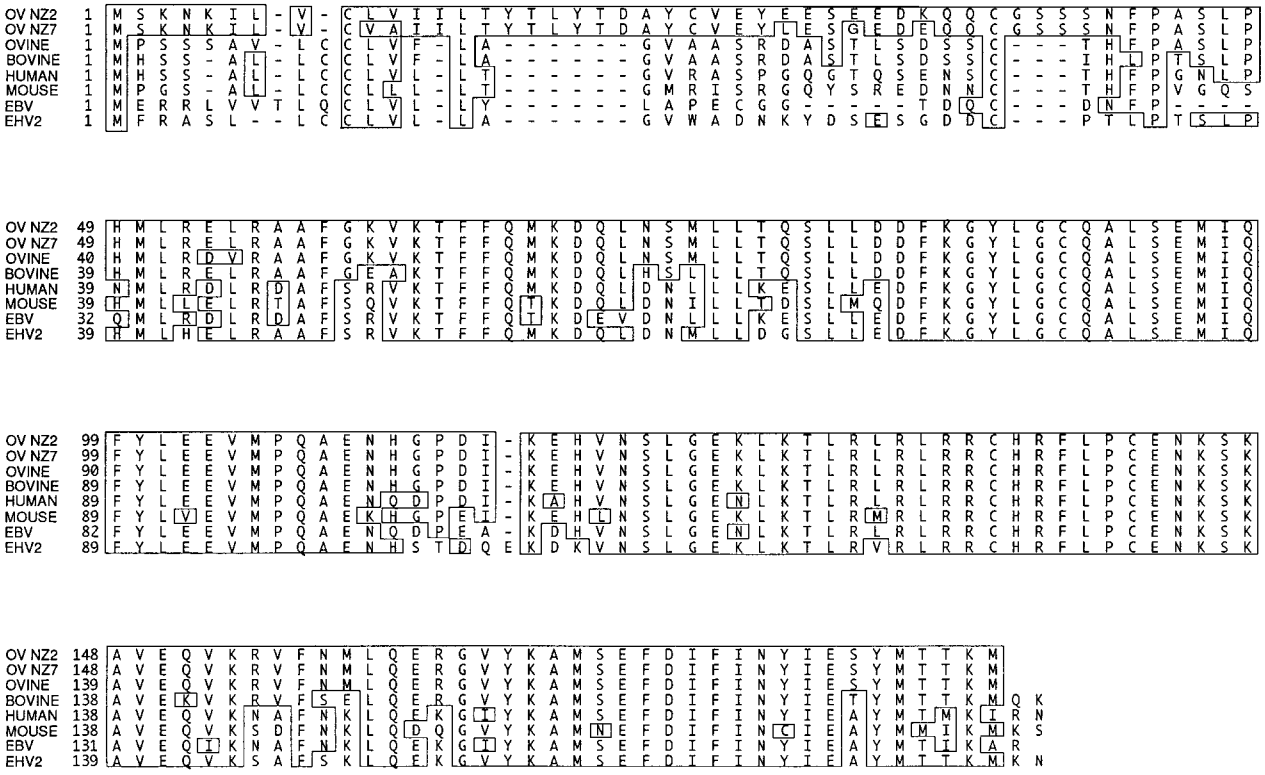

FIG. 3. Alignment of the inferred amino acid sequences of the OV NZ2-IL-10 and OV NZ7-IL-10 gene products with sequences of mammalian IL-10s and products of viral IL-10-like genes by using CLUSTAL (13). The mammalian IL-10s compared are ovine IL-10 (6), bovine IL-10 (12), human IL-10 (32), and murine IL-10 (20). The herpesvirus IL-10-like gene products are EBV IL-10 (20) and EHV2 strain T400/3 IL-10 (27). Amino acids identical to those of OV NZ2 IL-10 are boxed.

fected host. It is likely that the role of OV IL-10 is to defend infected cells from the primary immune responses of inflammation, cytolysis by natural killer cells, and apoptosis during the early stages of viral infection. Since OV replication is restricted to skin epithelial cells, viral IL-10 could suppress immunity within the infected site via a paracrine mechanism in which the virokine is secreted from infected cells into the surrounding tissue.

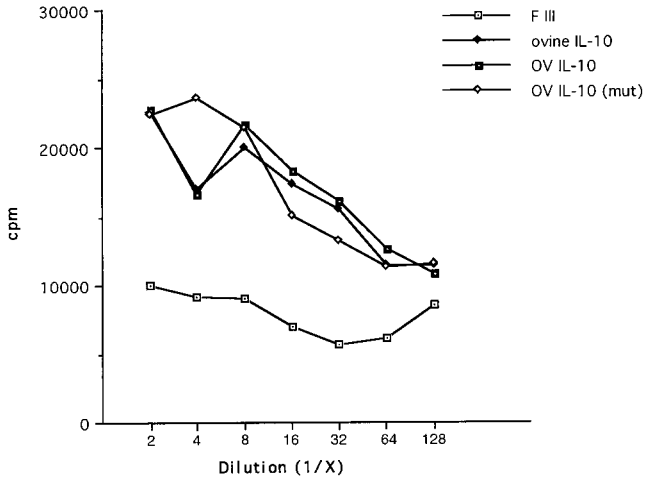

FIG. 4. Murine thymocyte proliferation analysis of OV-encoded IL-10. Dilution series of conditioned medium from COS cells, transfected with the plasmids shown below, were added to murine thymocytes. Counts per minute represent the incorporation of [^{3}H]thymidine during the final 18 to 24 h of a 120-h incubation. The results shown are the mean of duplicate determinations. FIII, FIII plasmid only (16); ovine IL-10, FIII plasmid containing the ovine IL-10 gene (16); OV IL-10, FIII plasmid containing the OV IL-10-like gene; OV IL-10 (mut), FIII plasmid containing a mutated IL-10-like gene.

A role for OV IL-10 in subversion of specific immunity is suggested by the fact that sheep are susceptible to reinfection with OV (17, 24). Cell-mediated immune responses, perhaps cytotoxic T cells, are thought to be critical in recovery from OV infection since humoral antibody appears to play no role (18). Recent studies suggest that sheep produce an aberrant cellular response to OV infection (10, 11). CD4 T cells were found at higher levels than CD8 T cells or B cells in lymph draining from the site of infection, particularly in the early stages of reinfection. It was expected that the CD8 T-cell response might have been numerically more significant if CD8 cytolytic cells were important in containing the virus, and the observation suggests that OV has acquired a mechanism for preventing the accumulation and activation of CD8 T cells. These findings are consistent with the effect of IL-10 on cellular immunity and analogous with the immune response observed in humans infected with EBV. Primary EBV infection in adults is associated with substantial dysfunction in both T- and B-cell compartments of the immune system (30). We postulate that OV IL-10 inhibits the induction of a Th1 response by a mechanism similar to that of BCRF1 and thus skews the host immune response towards an inappropriate Th2-like response.

The striking homology of OV IL-10 to ovine IL-10 strongly suggests that the viral gene represents a processed ovine gene captured by the virus at a late stage in its evolutionary development. Further studies are under way to characterize the

range of activities of OV IL-10 and to determine the effect of its gene on pathogenesis and immunity in the infected host.

Nucleotide sequence accession numbers. The sequences of OV NZ2-IL-10 and OV NZ7-IL-10 have been assigned GenBank accession no. U60552 and U82239, respectively.

REFERENCES

1. **Alcami, A., and G. L. Smith.** 1995. Cytokine receptors encoded by poxviruses: a lesson in cytokine biology. Immunol. Today **16:**474–478.
2. **Altshul, S. F., W. Gish, W. Miller, E. W. Meyers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
3. **Baer, R., A. T. Bankier, M. D. Biggin, P. L. Deininger, P. J. Farrell, T. J. Gibson, G. Hatfull, G. S. Hudson, S. C. Satchwell, C. Seguin, P. S. Tuffnell, and B. G. Barrell.** 1984. DNA sequence and expression of the B 95-8 Epstein-Barr virus genome. Nature **310:**207–211.
4. **Balassu, T. C., and A. J. Robinson.** 1987. Orf virus replication in bovine testis cells: kinetics of viral DNA, polypeptide, and infectious virus production and analysis of virion polypeptides. Arch. Virol. **97:**267–281.
5. **Buller, R. M., and G. T. Palumbo.** 1991. Poxvirus pathogenesis. Microbiol. Rev. **55:**80–122.
6. **Dutia, B. M., P. Hunt, D. R. Sargan, R. G. Dalziel, and J. Hopkins.** 1994. Sequence of the sheep interleukin-10-encoding cDNA. Gene **149:**393–394.
7. **Fleming, S. B., K. M. Fraser, A. A. Mercer, and A. J. Robinson.** 1991. Vaccinia virus-like early transcriptional control sequences flank an early gene in orf virus. Gene **97:**207–212.
8. **Fleming, S. B., A. A. Mercer, K. M. Fraser, D. J. Lyttle, and A. J. Robinson.** 1992. *In vivo* recognition of orf virus early transcriptional promoters in a vaccinia virus recombinant. Virology **187:**464–471.
9. **Fleming, S. B., J. Blok, K. M. Fraser, A. A. Mercer, and A. J. Robinson.** 1993. Conservation of gene structure and arrangement between vaccinia virus and orf virus. Virology **195:**175–184.
10. **Haig, D., D. Deane, A. Percival, N. Myatt, J. Thomson, L. Inglis, J. Rothel, Heng-Fong Seow, P. Wood, H. R. P. Miller, and H. W. Reid.** 1996. The cytokine response of afferent lymph following orf virus reinfection of sheep. Vet. Dermatol. **7:**11–20.
11. **Haig, D. M., G. Entrican, D. L. Yirrell, D. Deane, H. R. P. Miller, M. Norval, and H. W. Reid.** 1992. Differential appearance of interferon (and colony stimulating activity) in afferent lymph following orf virus infection in sheep. Vet. Dermatol. **3:**221–229.
12. **Hash, S. M., W. C. Brown, and A. C. Rice-Ficht.** 1994. Characterization of a cDNA encoding bovine interleukin 10: kinetics of expression of bovine lymphocytes. Gene **139:**257–261.
13. **Higgins, D. G., and P. M. Sharp.** 1988. CLUSTAL: a package for performing multiple sequence alignments on a microcomputer. Gene **73:**237–244.
14. **Innis, M. A., G. H. Gelfand, J. J. Sninsky, and T. J. White (ed.).** 1990. PCR protocols: a guide to methods and applications. Academic Press, San Diego, Calif.
15. **Lyttle, D. J., K. M. Fraser, S. B. Fleming, A. A. Mercer, and A. J. Robinson.** 1994. Homologs of vascular endothelial growth factor are encoded by the poxvirus orf virus. J. Virol. **68:**84–92.
16. **Martin, H. M., A. D. Nash, and A. E. Andrews.** 1995. Cloning and characterisation of an ovine interleukin-10-encoding cDNA. Gene **159:**187–191.
17. **McKeever, D. J., D. M. Jenkinson, G. Hutchison, and H. W. Reid.** 1988. Studies on the pathogenesis of orf virus infection in sheep. J. Comp. Pathol. **99:**317–328.
18. **Mercer, A. A., D. L. Yirrell, W. H. Reid, and A. J. Robinson.** 1994. Lack of cross protection between vaccinia virus and orf virus in hysterectomy-procured, barrier-maintained lambs. Vet. Microbiol. **41:**373–382.
19. **Mercer, A. A., D. J. Lyttle, E. M. Whelan, S. B. Fleming, and J. T. Sullivan.** 1995. The establishment of a genetic map of orf virus reveals a pattern of genomic organisation that is highly conserved among divergent poxviruses. Virology **212:**698–704.
20. **Moore, K. W., P. Vieira, D. F. Fiorentino, M. L. Trounstine, T. A. Khan, and T. R. Mosmann.** 1990. Homology of cytokine synthesis inhibitory factor (IL-10) to the Epstein-Barr virus gene BCRF1. Science **248:**1230–1234.
21. **Mosmann, T. R.** 1994. Properties and functions of interleukin-10. Adv. Immunol. **56:**1–25.
22. **Moss, B.** 1994. Vaccinia virus transcription, p. 185–205. *In* R. C. Conway and J. W. Conway (ed.), Transcription: mechanisms and regulation. Raven Press, New York, N.Y.
23. **Pickup, D. J.** 1994. Poxviral modifiers of cytokine responses to infection. Infect. Agents Dis. **3:**116–127.
24. **Robinson, A. J., and T. C. Balassu.** 1981. Contagious pustular dermatitis (orf). Vet. Bull. **51:**771–781.
25. **Robinson, A. J., G. Ellis, and T. Balassu.** 1982. The genome of orf virus: restriction endonuclease analysis of viral DNA isolated from lesions of orf virus in sheep. Arch. Virol. **71:**43–55.
26. **Rode, H., J. J. Bugert, M. Handermann, P. Schnitzler, R. Kehm, W. Janssen, H. Delius, and G. Darai.** 1994. Molecular characterization and determination of the coding capacity of the genome of equine herpesvirus type 2 between the genome coordinates 0.235 and 0.258 (the *Eco*RI DNA fragment N; 4.2 kbp). Virus Genes **9:**61–75.
27. **Rode, H. J., W. Janssen, A. Rosen-Wolff, J. J. Bugert, P. Thein, Y. Becker, and G. Darai.** 1993. The genome of equine herpes virus type 2 harbors an interleukin-10 (IL-10)-like gene. Virus Genes **7:**111–116.
28. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
29. **Spriggs, M. K.** 1996. One step ahead of the game: viral immunomodulatory molecules. Annu. Rev. Immunol. **14:**101–130.
30. **Thorley-Lawson, D. A.** 1988. Immunological responses to Epstein-Barr virus infection and pathogenesis of EBV induced diseases. Biochim. Biophys. Acta **948:**263–286.
31. **Turner, P. C., and R. W. Moyer.** 1990. The molecular pathogenesis of poxviruses. Curr. Top. Microbiol. Immunol. **163:**125–151.
32. **Vieira, P., R. De Waal-Malefyt, M.-N. Dang, K. E. Johnson, R. Kastelein, D. F. Fiorentino, J. E. DeVries, M.-G. Roncarlo, T. R. Mosmann, and K. W. Moore.** 1991. Isolation and expression of human cytokine synthesis inhibitory factor cDNA clones: homology to Epstein-Barr virus open reading frame BCRF1. Proc. Natl. Acad. Sci. USA **88:**1172–1176.