Haematology and Blood Transfusion Vol. 26
Modern Trends in Human Leukemia IV
Edited by Neth, Gallo, Graf, Mannweiler, Winkler

Identification of the Avian Myeloblastosis Virus Genome

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In addition to neoplasias caused in chickens by helper viruses of the avian myeloblastosis virus (AMV) complex, acute myeloblastic leukemia is induced by a defective leukemogenic component. To identify the leukemogenic viral genome the unintegrated and integrated viral DNA intermediates were chracterized. Linear viral DNA isolated from the cytoplasm of helper virus (MAV-1 or MAV-2) infected chicken embryonic fibroblasts (CEF) has a mass of 5.3 million daltons (md) (Bergmann et al. 1980). The linear MAV-1 and MAV-2 DNAs can be distinguished from one another by cleavage with the restriction endonuclease Hind III, because MAV-1 DNA contains one more Hind III recognition site than does MAV-2. Linear viral DNA isolated from the cytoplasm of CEF infected with standard AMV (AMV-S) which contains the defective leukemogenic component showed a minor molecular species of 4.9 md in addition to the major species of 5.3 md. Eco RI or Hind III digestion of the linear viral DNA from AMV-S-infected CEF generates specific fragments in addition to those unique for MAV-1 or MAV-2 viral DNA (Bergmann et al. 1980). A Hind III digest of AMV-S linear viral DNA also indicates that more than 90% of the AMV-S virus complex is MAV-1 like, while the remainder represents both the leukemogenic and MAV-2-like viruses (Souza et al. 1980).

The arrangement of endogenous proviral DNA in the chicken genome was determined prior to AMV infection. Infections were carried out both in vitro and in vivo using viral stocks of AMV-S, AMV-B, or AMV-C. (Souza and Baluda, to be published; Souza et al., to be published a). AMV-B contains only viruse of subgroup B, whereas AMV-S contains both subgroup A and B viruses. AMV-C originated from a clone of nonproducer myeoloblasts superinfected with tdB77 subgroup C. Peripheral blood leukemic myeloblasts induced by AMV contained two specific restriction enzyme fragments, an Eco RI 2.2 md and a Hind III 2.6 md, in addition to the endogenous proviral pattern. These two fragments were also detected in the appropriate digest of AMV-S linear viral DNA. DNA from 16 clones of leukemic myeloblasts isolated from AMV-converted yolk sac cell cultures contained the Eco RI 2.2 md and Hind III 2.6 md fragments, regardless of the endogenous complement or the pseudotype of AMV used for conversion. In addition, 2 of the 16 leukemic myeloblast clones contained the two leukemia-specific fragements in the absence of any detectable helper provirus (1). (Souza and Baluda, to be published; Souza et al., to be published a).

Juncture fragments between cellular and proviral sequences could be detected in the leukemic myeloblast clones, but were generally not seen in peripheral blood leukemic myeloblasts. The number of differently sized juncture fragments detected in the leukemic myeloblast clones indicated that the DNA intermediates of the AMV complex can integrate at multiple sites in host DNA (Souza et al., to be published a)

Recombinant DNA clones were constructed by inserting 16–20-kilobase fragments of Eco RI partially digested leukemic myeloblast DNA into phage λ Charon 4a (Soza et al. 1980, to be published b). Clones containing proviral sequences were identified by hybridization with ^{125}I-AMV-S RNA. Restriction enzyme analysis of one clone, λ11A1-1, showed that the proviral DNA is flanked by cellular sequences on either side, contains both the Eco

RI 2.2 md and Hind III 2.6 md leukemia specific fragments, and has a mass of approximately 4.9 md (Souza et al. 1980). This molecular mass is the same as that of an unintegrated linear viral DNA found in CEF infected by AMV-S but not in CEF infected by MAV-1 or MAV-2. Therefore, this provirus could represent the AMV genome responsible for acute myeloblastic leukemia. Another λ-chicken hybrid clone, λ10A2-1, contained 85% of a MAV-1-like genome starting from the 5′ end with respect to viral RNA. Comparison of the AMV genome from λ11A1-1 with the MAV-1-like genome from λ10A2-1 by restriction endonuclease mapping (Fig. 1) and

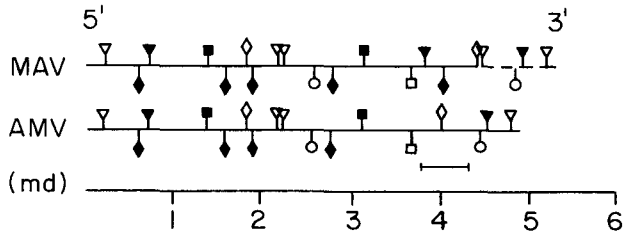

Fig. 1. Restriction enzyme maps of the presumptive AMV and MAV-1-like genomes. Restriction endonuclease sites are localized for the proviral DNA of the presumptive AMV provirus in λ-hybrid 11Al-1 and the partial MAV-1-like provirus in λ-hybrid 10A2-1. The *dashed line* in the MAV map indicates that part of the MAV-1-like genome not present in the λ10A2-1 hybrid. The location of the 3′ terminal enzyme sites in MAV-1 were determined with linear viral DNA. Enzyme sites: (▽) Hind III, (◇) Eco RI, (○) Xba I, (□) Kpn I, (◆) Bam HI, (■) Bgl II, and (▼) Xho I. The *bar* under the AMV map represents the region of cellular substitution

heteroduplex analysis indicated both genomes are homologous over 3.6 md from the 5′ terminus and lack homology on the 3′ side of the single Kpn I site in each genome (Souza et al., to be published a,b). R-loop analyses carried out with the λ-recombinant containing the AMV genome and 35S AMV-S RNA revealed two types of R-loops (Souza et al., to be published b). The first type showed the RNA hybridized over the entire length of the presumptive AMV genome, while the second type showed the RNA hybridzed for approximately 5700 bases from the 5′ end of the provirus, displaced by a DNA-DNA duplex for the next 900 bases, and hybridized again for the remaining 700 bases to the 3′ terminus. The first type is interpreted to represent an R-loop of AMV RNA and the second type, and R-loop of MAV RNA to the AMV genome in which a contiguous 900 base substitution exists.

Southern blots of Eco RI digested DNA from various strains of uninfected chickens were hybridized with either ^{125}I-labeled RAV-0, MAV-2, or AMV-S RNA. In addition to the Eco RI fragments containing endogenous proviral sequences detected by the MAV-2 or RAV-0 probe, two AMV-S-specific fragments of 3.7 and 1.5 md were detected by the AMV-S probe (Souza and Baluda 1978). ^{32}P-DNA probes prepared from the 3′ region of the AMV DNA genome were hybridized to Eco RI DNA blots prepared from various strains of uninfected chickens (Souza et al. to be published b). The ^{32}P-AMV probes detected the same cellular sequences, i.e., the same Eco RI fragments that were detected with ^{125}I-AMV-S RNA. AMV-specific sequences could also be detected in uninfected Peking duck Eco RI DNA blots using the ^{32}P-labeled AMV probes. Ducks do not contain endogenous proviral DNA homologous to the known avian retroviruses. A ^{32}P-labeled probe made from a similar region of the MAV-1-like provirus contained only homology to endogenous proviral sequences in chicken DNAs and no homology to the Peking duck DNA (Souza et al., to be published b). These hybridization studies have demonstrated that the AMV genome contains a cellular substitution. The physical mapping studies place this substitution in the region of the genome normally occuppied by the envelope gene. Thus, this cellular substitution in AMV may be responsible for its leukemogenic potential and its defectiveness.

AMV differs from avian defective transforming viruses such as MC-29, AEV, and MH2 in the following: (1) AMV is only slightly smaller than its natural helper(s), (2) the substitution if contains is half the size of the substitution contained in the other defective viruses, (3) the substitution in AMV appears to only have replaced the env gene, whereas the substitutions in the other defective viruses have replaced part of gag, pol, and env, and (4) AMV does not make an unprocessed transforming protein containing part of gag (Silva and Baluda, to be published). However, the AMV genome resembles physically that of Bryan RSV, a defective avian sarcoma virus.

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