Existence of a Reverse Transcriptase–p30 Complex in AKR Mice with a High Incidence of Spontaneous Lymphocytic Leukaemia

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SUMMARY

The virus proteins, reverse transcriptase (RT) and p30, were found to increase with time in the subcellular fractions of lymphocytic tissue from either the thymus or spleen of AKR mice with spontaneous lymphocytic leukaemia. Significant levels of RT activity were first detected in the microsomal fractions of the two tissues at 15 and 20 weeks old, respectively. Although low amounts of p30 could be found in both tissues within the first week of life, the overall increase in the amount of p30 within each tissue followed much the same course as that shown by RT. In addition, a protein complex consisting of p30 and RT was first found in thymus and spleen lymphocytes of 15 and 20 week-old animals, respectively. The complex increased in amount in both organs as the animals aged, reaching a maximum level in 30 week-old mice. Repeated attempts to detect other virus proteins such as gp70 in association with the complex by immunological means were unsuccessful. The complex could not be found in lymphocytic tissue taken from younger animals or in ‘non-target’ organs, such as liver or kidney, of animals with leukaemia. In animals treated with antiviral IgG, which delayed the development of spontaneous leukaemia, the complex did not appear until much later in life (45 weeks) and then in considerably smaller amounts.

INTRODUCTION

The high leukaemic AKR/J mouse regularly expresses significant levels of ecotropic virus (AKR type) early in life (Rowe & Pincus, 1972; Gross, 1974). Although this virus is infectious it is not until the animal is 5 or 6 months old that leukaemia is found (Gross, 1974). The AKR virus genome is transmitted in a stable heritable form and virus genetic material is present in the host cellular DNA (Chattopadhyay et al. 1974). Although the exact mechanism for leukaemogenesis is not known, it would appear that the mice develop spontaneous formation of a recombinant between ecotropic and xenotropic (X-tropic) virus at about 5 to 6 months old (Hartley et al. 1977) and that this recombinational event occurs within the envelope region (Elder et al. 1978).

The presence of a complex consisting of both reverse transcriptase (RT) and p30 has been demonstrated (Bandyopadhyay, 1977) from the virion of Rauscher leukaemia virus (RLV), a leukaemogenic virus (Rauscher, 1962). Because this complex has a greater efficiency for in vitro transcription of virus 70S RNA (Bandyopadhyay et al. 1976; Bandyopadhyay & Levy, 1978), it was postulated that the formation of the complex may be involved in the
assembly of the oncogenic virus (Bandyopadhyay et al. 1976; Bandyopadhyay, 1977; Bandyopadhyay & Levy, 1978). It was of interest, therefore, to determine whether the RT-p30 complex exists in AKR animals and, if so, what relationship it has with respect to the age of the mice and to the onset of their spontaneous leukaemia.

The work described here demonstrates that the complex does, indeed, exist and that its formation in the subcellular fractions of thymus and of spleen increases in amount as the animal ages, reaching a maximum level at 30 weeks old. In AKR mice treated with antiviral IgG, which delayed onset of disease (Fish et al. 1979), the expression of the complex was not only retarded, but its absolute amount was also reduced.

METHODS

Chemicals. Radioactive nucleotides were purchased from New England Nuclear (Boston, Mass., U.S.A.). Amino acids (radioactive and non-radioactive) and all marker RNAs (4S, 16S, 18S and 28S) were obtained from Schwartz-Bioresearch (Orangeburg, N.Y., U.S.A.). Unlabelled deoxynucleoside 5'-triphosphate, dithiothreitol, calf thymus DNA, yeast RNA and bovine serum albumin were products of Calbiochem (Los Angeles, Calif., U.S.A.). Sephadex G-200 and Sepharose 6B were purchased from Pharmacia (Uppsala, Sweden). Pancreatic DNase and pancreatic RNase were obtained from Worthington Chemicals (Freehold, N.J., U.S.A.). The synthetic polynucleotides were supplied by Collaborative Research (Waltham, Mass., U.S.A.). The isolation procedure for virus 70S RNA, 35S RNA and 4S RNA has been published elsewhere (Biswal & Benyesh-Melnick, 1969). RT and p30 were purified by sequential column chromatography as described previously (Deepak et al. 1975; Bandyopadhyay, 1977). Retroviruses were obtained from Program Resources and Logistic, National Cancer Institute, Bethesda, Md., U.S.A. and further purified in this laboratory by banding over a sucrose gradient.

Animals. AKR/J female mice were obtained either from Jackson Laboratory (Bar Harbor, Maine, U.S.A.) or from the Frederick Cancer Research Center (Frederick, Md., U.S.A.).

RT assay. DNA polymerase activity was measured by the incorporation of 3H-dTMP or 3H-dGMP into 20 µg/ml of either (dT)$_{12}$-(rA)$_n$ or (dG)$_{12}$-(rC)$_n$, as described earlier (Bandyopadhyay, 1977).

Cellular DNA polymerase assay. DNA polymerase-α, DNA polymerase-β and DNA polymerase-γ were assayed as described by others (Bollum, 1967; Weissbach, 1977).

Lymphocyte isolation. Lymphocytes were isolated either from the thymus or spleen following the procedure of Jaffe et al. (1974). In each case, thymuses or spleens of 10 mice were pooled and the lymphocytes were isolated.

Fractionation of lymphocytes. Subcellular fractionation of the lymphocytes by differential centrifugation was performed as described previously (Bandyopadhyay, 1975). The procedure, described here briefly, was carried out at 0 to 4°C.

Nearly 1 g of cells (wet wt.) was suspended in 15 ml 10 mm-tris-HCl buffer (pH 7.2) containing 2 mm-dithiothreitol and 10% glycerol (buffer A). After homogenization of the mixture in a Dounce homogenizer with 30 strokes using a tight type B pestle, solid sucrose was added to 10 ml of the homogenate to a final sucrose concentration of 0.35 M. Cellular debris was removed from the homogenate by centrifugation at 1000 g for 10 min, after which the supernatant was centrifuged at 12000 g for 15 min to sediment mitochondria. The supernatant, free of nuclear and mitochondrial substances as shown by light microscopy and enzyme markers (cytochrome oxidase, lactate dehydrogenase and DNA-directed RNA polymerase), was then centrifuged at 105000 g for 120 min. The supernatant retained from this centrifugation was used as the cytosol fraction.
The pellet, suspended in 5 ml of buffer A, was layered on to 12 ml of a 20 to 55% sucrose gradient and centrifuged in an SW41 rotor at 105000 g for 16 h in a Beckman L3-65B centrifuge. The gradient was fractionated and the fractions within the 1.14 to 1.20 g/ml density region were combined and diluted fivefold with buffer A. Glycerol was then slowly added to the buffer A-diluted material to bring the glycerol concentration to 20% (v/v). The mixture was subjected to centrifugation at 105000 g for 3 h and the pellet which formed was used as the microsomal fraction.

For solubilization, the microsomal pellet was resuspended in 1 ml of buffer A containing 1% Triton X-100 and 1 mM-KCl. After gentle stirring at 0°C for 1 h the suspension was diluted fourfold with buffer A and centrifuged at 27000 g for 15 min. The supernatant fluid was dialysed against two 500 ml changes of buffer A and used as a solubilized microsomal fraction. The final concentration of Triton X-100 in the microsomal fraction was maintained at 1% by adding concentrated Triton X-100 before each assay.

Purification of virus antigens from AKR-type virus. AKR ecotropic virus (30 mg), after two cycles of freezing and thawing, was centrifuged at 105000 g for 60 min. The gp70 was purified from the supernatant solution by column chromatography on phosphocellulose and Sephadex G-100 (Strand & August, 1973).

The virus pellet was resuspended in 10 ml of a solution containing 10 mM-tris-HCl buffer (pH 7.2), 20 mM-dithiothreitol, 0.2 M-KCl and 1% Triton X-100. RT, p30 and p15 were purified by column chromatography as described previously (Deepak et al. 1975; Bandyopadhyay, 1977). Evidence for protein purity was the presence of a single band on SDS–PAGE (Deepak et al. 1975; Bandyopadhyay, 1977).

Radioimmunoassay and radioimmunoprecipitation of virus antigens. Virus antigens, purified as described above, were labelled with 125I so that the amount of radioactivity was between 10^4 and 10^5 cpm/min/ng protein (Hunter, 1967). Quantitative analysis of the antigens was performed as described by Strand & August (1973). Rabbit antiserum made against purified p30 from AKR ecotropic virus (anti-AKR p30), which precipitates only p30 and no other virus proteins, was used to detect and quantify p30. Rabbit antiserum made against the Triton X-100-disrupted whole AKR virus was used to assay for gp70 or p15. Radioimmunoprecipitation (RIP) of virus antigens was performed as described by Jamjoom et al. (1977). The rabbit antiserum, made against purified RT from AKR ecotropic virus (anti-AKR-RT), was absorbed with p30 and p10. This antiserum is capable of precipitating only RT in RIP.

Passive immunization of AKR/J mice with various antibodies. AKR/J mice were immunized with several antiviral IgG preparations. The prevention of spontaneous leukaemia in these mice as a result of this passive immunization has been described previously (Huebner et al. 1976; Fish et al. 1979). Several groups of AKR mice (70 in each group) were immunized with different IgG preparations. The immunization schedule was as follows: day 0, 0.05 ml; day 3, 0.05 ml; day 7, 0.05 ml; day 10, 0.1 ml; day 14, 0.1 ml; day 17, 0.1 ml; day 27, 0.1 ml; day 24, 0.1 ml; day 28, 0.1 ml; and day 31, 0.1 ml.

RESULTS

Determination of RT activity and p30 in thymus and spleen

From the work of Metcalf (1966), it is known that AKR mice develop lymphocytic leukaemia initially in the thymus and secondarily in the spleen. Since these tissues are apparent ‘target’ organs, the subcellular fractions of the lymphocytes of both tissues were examined for RT activity. Although AKR ecotropic virus is known to be present in these animals within the first week of their lives (Rowe, 1972; Rowe & Pincus, 1972), no ^3H-TMP incorporation into (dT)_{12}-(rA)$_n$ was detected in the microsomal or cytosol fractions of either
Fig. 1. Increase in (a) RT and (b) p30 activities as a function of age in the microsomal and cytosol fractions of lymphocytic tissue of the AKR/J mice. Subcellular fractions taken from purified lymphocytes of the thymus and spleen were prepared as described in Methods. RT activity using \(^{3}\text{H}-\text{dTMP}\) as substrate was also measured as described in Methods, whereas p30 activity was measured by radioimmunoassay using rabbit antiserum made against p30 purified from AKR virus (Strand & August, 1973). ●—●, ○—○, Microsomal fraction of thymus and splenic lymphocytes, respectively; ▲—▲, △—△, cytosol fraction of thymus and splenic lymphocytes, respectively. Since the subcellular fractions of thymus and spleen have a small amount of cellular DNA-polymerizing activity (DNA polymerase-α and DNA polymerase-β; see text), about 20% of the total enzyme activity obtained in the assay described above was routinely deducted from all measurements. The corrected results are those shown in the figure.

organ until the mice were 12 weeks old (Fig. 1a). Then, significant enzyme activity was detected in the microsomal fractions of thymus and splenic lymphocytes (at 15 and 20 weeks old, respectively). In the cytosol fractions of both tissues, on the other hand, enzyme activity was not detected until somewhat later (i.e. at 20 and 24 weeks old within the thymus and spleen, respectively). In all cases, enzyme activity increased as the animals aged (Fig. 1a).

With respect to p30, although low amounts of this protein could be found soon after the birth of the mice, the overall increase in the amount of p30 within each tissue followed much the same course as that shown by RT (Fig. 1b). A similar type of elevation of p30 from thymus was also reported elsewhere (Kwaeshima et al. 1976).

**Evidence that the DNA-polymerizing activity is mainly RT**

Since the \((\text{dT})_{12}(\text{rA})_{n}\) polymerizing activity described above is measured in relatively crude extracts obtained from subcellular fractions of thymus and spleen, it was important to establish unequivocally the nature of the enzyme being studied. When the enzymic activity in these fractions was assayed for \(^{3}\text{H}-\text{dGMP}\) incorporation into \((\text{dG})_{12}(\text{rC})_{n}\),
similar increases in activity to that observed for $^3$H-dTMP incorporation into (dT)$_{12}$.($rA$)$_n$ could be seen. Furthermore, if enzyme activity was assayed in the presence of IgG purified from rabbit antiserum made against AKR virus RT about 60\% of the DNA-polymerizing activity at 15 weeks old is inhibited. As the animal aged the inhibition rose to 80\% and remained at that level throughout the study.

If, in addition, those subcellular fractions containing the polymerizing enzymes are subjected to phosphocellulose column chromatography, three DNA-polymerizing enzymes could be detected (Fig. 2). The first, eluted from the column at 0·1 M-KCl, decreases in activity as the animal ages. Its mol. wt. is 160000 and the efficiency by which it catalyses the incorporation of $^3$H-dTMP into (dT)$_{12}$.($dA$)$_n$ is very high. Based on similarities in these properties to those published for DNA polymerase-\(\alpha\) (Weissbach, 1977), this activity has, in fact, been designated DNA polymerase-\(\alpha\).

The third activity (Fig. 2), has been called DNA polymerase-\(\beta\) since it resembles that enzyme in mol. wt. (35000), in the very high efficiency of incorporation of $^3$H-dTMP into (dT)$_{12}$.($dA$)$_n$ and in the molarity of KCl (0·5 M) needed to elute it from the phosphocellulose column (Weissbach, 1977). It should be noted that its activity remains essentially unchanged throughout the course of the study.

The second or intermediate activity increases as the animal ages and its elution at 0·3 M-KCl corresponds to that molarity of KCl needed to elute RT from this column. It is, moreover, inhibited completely by IgG purified from rabbit antiserum made against AKR ecotropic virus RT whereas the two other enzymes, designated DNA polymerase-\(\alpha\) and DNA polymerase-\(\beta\) are not inhibited by the same antibody. Furthermore, similar activity was not detected in non-infected cells, e.g. JLS-V9 or Balb 3T3 cells.
Another cellular DNA polymerase activity (DNA polymerase-\(\gamma\)) known to be present in eukaryotic cells (Weissbach, 1977) could not be detected in the microsomal or cytosol fractions of either the spleen or thymus but could be detected in the nuclear fractions of both tissues. This activity is also not inhibited by IgG purified from rabbit antiserum made against AKR ecotropic virus RT.

In addition, none of the DNA polymerases (\(\alpha\), \(\beta\) or \(\gamma\)) are able to form a stable complex with \(p30\), purified from AKR ecotropic virus. The enzyme activity eluted at \(0.3 \text{ M-KCl}\) readily does so and the complex formed resembles one formed between \(p30\) and RT when both proteins are purified directly from AKR virus (Bandyopadhyay & Levy, 1979). We conclude that the enzyme eluted at \(0.3 \text{ M-KCl}\) is RT.

Finally, to eliminate the remote possibility that the second peak of enzyme activity (Fig. 2) may be a terminal transferase, all the subcellular fractions from thymus or spleen were assayed using, as a primer, the oligonucleotide most characteristic of terminal transferase activity, i.e. \((dG)_{12}\). Up to 10 weeks old, a slight amount of this activity could be detected but only in the microsomal fractions of the thymus. After this period, terminal transferase activity was not observed in the microsomal or cytosol fractions of thymus or spleen.

**Detection of an RT–\(p30\) complex in thymus and spleen**

In earlier studies, the existence of an RT–\(p30\) complex in RLV was shown after subjecting lysed virus to glycerol-gradient centrifugation (Bandyopadhyay, 1977) and demonstrating that the complex migrated as a faster moving (or heavier) component within the gradient than either RT or \(p30\) alone. When a lysed portion of microsomal fractions obtained from thymus lymphocytes at various time intervals was subjected to centrifugation on glycerol gradients for 2.5 h, RT was resolved into lighter and heavier activities (Fig. 3). The lighter component (fractions 24 to 27) migrated at the same position in the gradient as does RT itself, whereas the heavier one (fractions 13 to 18) was located at a position in the gradient comparable to that of the complex between RT and \(p30\) of RLV (Bandyopadhyay, 1977). If centrifugation was conducted for a longer period of time (e.g. 6 h), the complex sediments to the bottom of the gradient and RT migrates from the top to a position approximately at fraction 16 of Fig. 3. When each fraction within the gradient was examined for \(p30\) (Fig. 3), a slower and a faster moving component were seen: the former (fractions 26 to 28) corresponding to the migration of \(p30\) protein itself and the latter (fractions 13 to 18) corresponding to the complex between \(p30\) and RT. Similar to that noted above for RT, longer centrifugation (6 h) will allow \(p30\) to sediment further into the gradient to a position corresponding to fractions 20 to 23.

As might be anticipated from the inability to detect significant RT activity in the mouse thymus before 15 weeks old (Fig. 1a), the formation of the protein complex is clearly time dependent. Thus, as shown in the first panel of Fig. 3 (T-15), the existence of the complex is barely discernible in tissue taken from animals 15 weeks old. With advancing age, however (Fig. 3; T-20, T-25 and T-30), the amount of the complex present (in terms of RT activity) in the microsomal fraction of the thymus lymphocyte increases from an initial value of 2 pmol/mg microsomal protein until a maximum value of 70 pmol/mg microsomal protein is reached at the end of 30 weeks (Fig. 3; T-30).

Although the amount of complex present increases as the animals age, the ratio of the two components within the complex remains relatively unchanged. In the microsomal fractions of the thymus, for example, if ng of \(p30\):activity of RT in pmol is taken as a measure of the ratio between the two proteins, then over a 20 week period this ratio averages out to about 7:1. This is comparable to the result obtained for RLV in which the ratio of \(p30\):RT activity within the complex was about 8:1 (Bandyopadhyay, 1977; Bandyopadhyay & Levy, 1978).
Fig. 3. Changes with time in the amount of the RT-p30 complex present in microsomal fractions of thymus lymphocytes of AKR/J mice. Microsomal fractions were isolated from approx. 10^9 thymus lymphocytes as described in Methods. The microsomal fractions were resuspended in 1 ml 0.02 M-tris-HCl buffer (pH 7.6) containing 0.14 M-KCl, 10 mM-dithiothreitol and 0.05% Triton X-100. The disrupted microsomes were kept at 0 °C for 30 min and were then subjected to centrifugation at 27000 g for 10 min. The supernatant fluid (in each case containing 1 mg protein) was layered over 4.5 ml of a glycerol gradient (5 to 20%) in 10 mM-tris-HCl buffer (pH 7.6) containing 0.1 M-NaCl. After centrifugation at 60000 rev/min in an SW65 rotor for 2.5 h, the bottom of the tube was punctured and approx. 30 fractions, 200 µl in vol., were collected. RT activity in each fraction was measured with 20 µl samples using (dT)_{12}-(rA)_n as a primer-template (Bandyopadhyay, 1977). The p30 was measured by radioimmunoassay using rabbit antiserum made against p30 purified from AKR virus (Strand & August, 1973). ○—○, RT activity; ○—○, p30 activity. T-15, T-20, T-25 and T-30 are the microsomal fractions of lymphocytic tissue (of thymus) isolated from 15, 20, 25 and 30 week-old AKR/J mice, respectively. RNA markers (arrows) used to establish the sedimentation values within the gradient were subjected to simultaneous centrifugation under similar conditions.

Although the results described above for complex formation were obtained using thymus lymphocytes as the tissue source, the picture that emerged with the microsomal fractions of splenic lymphocytes was much the same, except that the complex could be detected only after about 20 weeks of life rather than the 15 weeks seen in the thymus. Analysis of the cytosol fractions of lymphocytes from either thymus or spleen indicated that the earliest time the complex could be detected was 24 weeks in thymus and 30 weeks in spleen and the complex levels in each case are very low.

Is the observed RT-p30 complex an artefact?

Since p30 has been shown to contain some hydrophobic regions (Swanson et al. 1978) it is conceivable that the protein may be associated with membrane components within the microsomal fractions and because of incomplete solubilization during the isolation procedure, p30 held by membrane constituents could somehow complex with any available RT.
Because this would imply that the complex was merely an artefact of isolation with little biological significance, it was important to examine the physical characteristics of the complex both for consistency and for possible relationships to similar complexes isolated from known tumour viruses.

The complex, isolated at weekly intervals beginning with 15 week-old mice, was radio-labelled with $^{125}$I and was then subjected to radioimmunoprecipitation using rabbit antiserum made against either AKR-RT or AKR-p30 and the precipitate was analysed by SDS–PAGE. Examination of autoradiograms of the gels revealed that in every case, irrespective of the animal's age, the protein bands of RT and p30 were seen. These results are similar to those found for the RT–p30 complex isolated from RLV or RLV-infected cells (Bandyopadhyay, 1977; Jamjoom et al. 1977).

In addition, the complex, irrespective of the age of the mouse from which it was isolated, could only be dissociated in KCl that was at least 0.8 M, suggesting involvement of ionic bonds between the components of the complex. Under these conditions, two proteins were released after dissociation which, upon analysis by glycerol-gradient centrifugation, migrated as p30 and as RT. Enzymic analysis and radioimmunoassay confirmed the identity of both proteins. This finding was similar to that observed in complexes isolated from RLV (Bandyopadhyay et al. 1976; Bandyopadhyay, 1977). Similar, too, were the observations that in no case would p30 or RT obtained from any of the sources described above bind to such common cellular proteins as bovine serum albumin, ovalbumin, chicken albumin, rabbit albumin, normal rabbit IgG or RNase A. Attempts to bind either protein to virus proteins such as gp70 or p15 purified from RLV were also unsuccessful.

It was concluded that the evidence described above does not suggest a random series of events in which, because of isolation techniques, two proteins that happened to be near enough to bind to each other, did so. On the contrary, the consistency of the physical data over an extended period of time, as well as their similarity to known tumour virus complexes, suggests that the complex is not an artefact of the isolation procedure.

**Absence of gp70 in the RT–p30 complex**

Although it is known that gp70 can be found in the serum and thymus lymphocytes of mice from the 24th week of age (Nowinski & Doyle, 1977), repeated attempts to detect this protein, in association with the complex in animals 30 weeks old, were unsuccessful.

**Absence of RT–p30 in other organs**

Attempts to detect the existence of the complex in subcellular fractions from organs of AKR mice, such as liver or kidney, were unsuccessful. Although all these animals develop leukaemia at about 28 weeks old, there is no suggestion of the presence of the complex in tissues other than thymus and spleen during the life span of the mice.

**Is the RT–p30 complex present in a non-oncogenic mouse tropic virus?**

Earlier studies indicated that RT has a strong affinity for p30 and that the complex formed between the two proteins could be isolated not only from RLV but also from RLV-infected cells (Bandyopadhyay et al. 1976; Bandyopadhyay, 1977; Jamjoom et al. 1977). To determine if the same type of complex could be found in either an N-tropic virus (e.g. AKR ecotropic or Balb-1 virus) or an X-tropic virus [e.g. NZB or Balb-2 virus (Levy, 1973; Todaro et al. 1973)] these viruses were lysed and the lysed material subjected to glycerol-gradient centrifugation. For comparative purposes, an oncogenic virus [Moloney leukaemia
Multiprotein complex in leukaemia

virus (MLV) was also examined for the presence of the complex. To give some degree of quantification to these studies, equal amounts (500 pmol) of RT activity from each virus were placed on a glycerol gradient. With respect to p30, although 7 µg of this protein, obtained from both the N-tropic and X-tropic viruses, was subjected to glycerol-gradient centrifugation, only 4 µg of MLV p30 was used for layering on the gradient. In the latter case, use of any additional amount of p30 had the effect of also increasing the amount of MLV RT activity beyond 500 pmol. This, of course, was quite suggestive that the two proteins (i.e. p30 and RT) were tied or in some way complexed to each other, a view which was later confirmed by the gradient profile obtained after centrifugation (Fig. 4). As is apparent from Fig. 4, in neither the N-tropic nor the X-tropic virus was the complex found, whereas it is readily demonstrable in lysates from the MLV.

In a quantitative sense the recovery of RT activity from either the N-tropic or X-tropic virus was at no time greater than 60% (300 pmol) of the initial activity placed on the gradient (Fig. 4, top two panels). This loss of activity is consistent with our experience of the enzyme, namely that RT can lose up to 40% of its activity when as a solitary protein it is subjected to glycerol-gradient centrifugation. With respect to p30, however, in both cases approx. 6.8 µg, or nearly 100% of the protein, could be recovered near the top of the gradient, indicating in effect that there is no detectable complex formation with RT.

In contrast to these results, of the 500 pmol of MLV RT subjected to centrifugation almost all of the activity was recovered either in the complex (35%; fractions 12 to 16) or as the free enzyme at the top of the gradient (65%). Similarly, with p30, approx. 100% of the protein (3.8 µg) was recovered either complexed to RT or as the free protein near the top of the gradient (Fig. 4, bottom panel).

Reduction in amount and delay in the formation of the complex in animals immunized with antiviral IgG

AKR/J mice were treated at birth with IgG preparations obtained from goats that had been either immunized with various murine leukaemia viruses (Huebner et al. 1976; Fish et al. 1979) or received no immunization at all. Such passive immunization procedures have been shown to delay spontaneous leukaemia in AKR/J mice when IgG having a high neutralizing titre against the ecotropic (AKR type) virus is administered. On the other hand, no protection is found when IgGs are used which have either low neutralizing titres against the ecotropic virus and/or high neutralizing titre against only the murine xenotropic virus (Huebner et al. 1976; Fish et al. 1979). Mice which had been treated with these various IgG preparations were examined at different time intervals for the presence of the RT–p30 complex (Table 1). In the first group which was either not treated or treated with anti-xenotropic IgG material, complex formation was observed in all animals at 20 weeks. This was followed by a rapid elevation in levels until a maximum was reached at about 35 weeks. The second group received anti-Gross leukaemia virus (anti-GLV) IgG and complex formation was not only delayed in appearance but the maximum amount of complex formed was much lower. In the last group which received anti-radiation leukaemia virus IgG, very little complex formation was seen and, when it did occur, was observed very late in the animal’s life.

DISCUSSION

In earlier studies with an oncogenic virus (RLV) it was demonstrated that the efficiency of transcription of virus 70S RNA by RT was increased several-fold when the enzyme was complexed to the major internal protein, p30 (Bandyopadhyay, 1977; Bandyopadhyay & Levy, 1978). This, coupled with the actual isolation of an RT–p30 complex from the virion
Fig. 4. Absence of p30-RT complex in N-tropic and X-tropic viruses. Ten µl 10% Triton X-100 were added to 5 ml (1 mg/ml protein) of five different viruses: N-tropic (Balb-1 and AKR), X-tropic (Balb-2 and NZB) and Moloney leukaemia virus (MLV), in the presence of 1 mM-dithiothreitol and 0.14 M-KCl. The disrupted virion preparation was kept on ice for 15 min and then centrifuged at 27000 g for 10 min. A sample of the supernatant solution from the N-tropic and X-tropic viruses (each containing 500 pmol RT activity and 7 µg p30) was then layered on to four glycerol gradients (5 to 20%) in 10 mM-tris-HCl buffer (pH 7.4) and 0.1 M-NaCl, whereas the sample from the supernatant solution of the MLV, although containing 500 pmol RT activity, had only 4 µg p30. This too was layered on to a glycerol gradient of the same composition as above. After centrifugation at 60000 rev/min in an SW65 rotor for 2.5 h, fractions (200 µl) were collected from the bottom of the tube. RT activity using (dT)12-16 (rA)6 was measured as described in Methods and p30 activity was measured by radioimmunoassay as described by Strand & August (1973). ●—●, RT; ○—○, p30. Although not shown, 4S and 16S RNA markers centrifuged simultaneously with the experimental material sedimented at fractions 28 and 15, respectively. Since the results of the centrifugation of the Balb-1 and Balb-2 virion preparations were similar to those obtained with the AKR and NZB preparations, only the latter two are shown.

itself or virus-infected cells, suggested to us that complex formation may be significant for oncogenic viruses (Bandyopadhyay et al. 1976; Jamjoom et al. 1977). In the present study, an extended examination of the protein complex in the AKR system has been made. From this work it is possible to note three significant findings with respect to complex formation in mouse tissue.

First, a p30–RT complex was isolated from the subcellular fractions of thymocytes and splenocytes of AKR/J mice starting at 15 to 20 weeks old. The complex, devoid of envelope protein gp70, was found to increase in amount as the mice aged, reaching a maximum in 30 week-old animals when the clinical symptoms of leukaemia can first be noted. Samples of the complex taken from both thymus and spleen as the animals aged show a constant ratio, (between p30 and RT) of approx. 7 ng of p30/pmol of 3H-dTMP incorporated. It should be noted that this is the same ratio as that found in the complex isolated from RLV (Bandyopadhyay, 1977). Complex formation could not be detected in non-target organs (liver or kidney) of AKR mouse at time intervals comparable to those in which it was found in thymus and in spleen.

Second, although genetic information for non-oncogenic viruses (both ecotropic and xenotropic) are known to be present in AKR mouse tissue (Chattopadhyay et al. 1974;
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* Animals were immunized with various IgG preparations as described by Fish et al. (1979).
† Reverse transcriptase (RT) activity is expressed in pmol of [H]-dTMP incorporation into (dT)12-(rA)6 per 60 min (Bandyopadhyay, 1977); p30 is expressed in ng of protein as determined by radioimmunoassay (Strand & August, 1973).
‡ RT-p30 complex was isolated by glycerol-gradient centrifugation from the subcellular fractions of various thymocytes as described in Fig. 3.
§ AD, Animals died before determinations could be made.
Kwaeshima et al. (1976) an RT–p30 complex could not be isolated from such non-oncogenic viruses (Fig. 4). In contrast, the complex can easily be obtained from such oncogenic viruses as RLV or MLV.

Third, a comparison of the relative stability in salt solutions of the complex actually isolated from AKR mouse tissue with that of p30–RT complexes indicated that the protein complex obtained from AKR mouse tissue is considerably more stable than might be expected from the union of autologous p30 and RT (Bandyopadhyay & Levy, 1979). In this regard, it has been shown recently that p30 from a xenotropic virus, when combined with RT from an N-tropic virus, gives rise to a far more stable complex than if either protein combined with its autologous antigen. The xenotropic p30 complexed with N-tropic RT is stable in KCl up to 0.8 M whereas the complex made up of autologous antigens (p30 and RT) dissociates at 0.4 M-KCl. The stability of the complex isolated from AKR mouse tissue resembles that of the non-autologous union in its resistance to salt dissociation, suggesting that the RT–p30 complex isolated from AKR thymocytes and splenocytes is a product of non-autologous origins.

In several related studies, Troxler et al. (1977) have shown that the spleen focus-forming virus behaves as if it were a recombinant between ecotropic and xenotropic virus (Hartley et al. 1977; Elder et al. 1978). Also, Hass (1978) has recently shown that the leukaemogenic activity of the radiation leukaemia virus isolates is dependent upon recombinational events taking place between endogenous ecotropic and xenotropic viruses, probably in the gag region (Benade et al. 1978). Whether such recombinants can produce a stable RT–p30 complex is not yet known, but in view of the resemblance of the RT–p30 complex reported in this paper, to one of non-autologous origin (xenotropic p30 bound to ecotropic RT) the question can be raised whether such a complex can be formed by virus resulting from recombinational events between ecotropic and xenotropic viruses.

Although the existence of the complex in AKR mice cannot, on an absolute basis, be related to the development of leukaemia, it is interesting to note that delay in the onset of the disease is accompanied by delay in the formation of the complex. In this connection it was found that animals treated with several IgG preparations other than those containing anti-xenotropic material, as well as untreated control mice, all showed the appearance of the complex before they were 20 weeks old and all developed leukaemia at the expected time. In contrast, in mice treated with anti-ecotropic virus IgG (GLV), complex formation could not be demonstrated until after 20 weeks of age and the level of complex formation was greatly reduced. The mice, in addition, did not develop their expected leukaemia either at the same rate or to the same extent (Table 1) as control animals. This phenomenon was even more pronounced in mice treated with anti-radiational leukaemia virus (anti-Rad LV) IgG (or anti-recombinant virus IgG). In these animals complex formation is not detected until 45 weeks and is found to increase in amount very slowly compared to the other groups of animals. The expression of spontaneous leukaemia is much more delayed in these mice.

REFERENCES


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