Retroviral Transformation In Vitro of Chicken T Cells Expressing Either α/β or γ/δ T Cell Receptors by Reticuloendotheliosis Virus Strain T

By Mina D. Marmot, Tania Benatar, and Michael J. H. Ratcliffe

From the Department of Microbiology and Immunology, McGill University, Montreal H3A 2B4, Canada

Summary

Exposure of normal juvenile chicken bone marrow cells to the replication defective avian reticuloendotheliosis virus strain T (REV-T) (chicken syncitial virus [CSV]) in vitro resulted in the generation of transformed cell lines containing T cells. The transformed T cells derived from bone marrow included cells expressing either α/β or γ/δ T cell receptors (TCRs) in proportions roughly equivalent to the proportions of TCR-α/β and TCR-γ/δ T cells found in the normal bone marrow in vivo. Essentially all TCR-α/β-expressing transformed bone marrow-derived T cells expressed CD8, whereas few, if any, expressed CD4. In contrast, among TCR-γ/δ T cells, both CD8+ and CD8- cells were derived, all of which were CD4-. Exposure of ex vivo spleen cells to REV(T(CSV)) yielded transformed polyclonal cell lines containing >99% B cells. However, REV(T(CSV)) infection of mitogen-activated spleen cells in vitro resulted in transformed populations containing predominantly T cells. This may be explained at least in part by in vitro activation resulting in dramatically increased levels of T cell REV(CSV) receptor expression. In contrast to REV(T(CSV))–transformed lines derived from normal bone marrow, transformed lines derived from activated spleen cells contained substantial numbers of CD4+ cells, all of which expressed TCR-α/β. While transformed T cells derived from bone marrow were stable for extended periods of in vitro culture and were cloned from single cells, transformed T cells from activated spleen were not stable and could not be cloned. We have therefore dissociated the initial transformation of T cells with REV(CSV) from the requirements for long-term growth. These results provide the first demonstration of efficient in vitro transformation of chicken T lineage cells by REV(T(CSV)). Since productive infection with REV(CSV) is not sufficient to promote long-term growth of transformed cells, these results further suggest that immortalization depends not only upon expression of the v-rel oncogene but also on intracellular factor(s) whose expression varies according to the state of T cell physiology and/or activation.

Reticuloendotheliosis virus strain T (REV-T)1 is a replication-defective avian retrovirus, first characterized as inducing acute reticuloendothelial neoplasia in vivo (1, 2). REV contains the transforming v-rel oncogene inserted within its env gene (3, 4), although the molecular basis for cell transformation by v-rel is currently unclear. REV(T) requires a helper virus for replication, the most widely used of which has been the REV-A retrovirus (5). However, REV-A itself is suppressive for lymphoid cells in vivo (6, 7), and infection in vivo or in vitro with REV-T(REV-A) has led to transformation of target cells frequently defined as being immature cells of hematopoietic origin (8–10). Subsequently, chicken syncitial virus (CSV) has been used as a helper virus for REV-T, and the resulting REV(T(CSV)) has proved to be highly efficient in the induction of lymphocyte transformation in vivo (11, 12) or in vitro (13, 14). Exposure of chickens in vivo leads to the rapid induction of polyclonal B cell tumors that can readily be adapted to in vitro growth (11, 12). Similarly, exposure of lymphoid cells in vitro to REV(CSV) also leads to polyclonal B cell transformation (13). While some reports have suggested that REV(T(REV-A))–transformed B lineage cells may undergo loss or changes within the Ig loci during in vitro culture (15–17), REV(T(CSV))–transformed B lineage clones induced in vivo or in vitro have stable Ig loci during extended periods of in vitro growth (11, 13). Recently, exposure of B cell–deficient chickens to REV(T(REV-A)) in vivo led to the generation of T cell tumors in vivo (18), suggesting that REV(T)-based viruses might provide a means of transforming at least some subsets of chicken T cells.

Avian species have provided valuable models for studies

---

1 Abbreviations used in this paper: CSV, chicken syncitial virus; REV, reticuloendotheliosis virus strain.
of lymphoid cell development from immature precursors largely as a consequence of the accessibility of the embryo to surgical manipulation (e.g., reference 19). The degree of conservation of T cell developmental biology (20, 21) and cell surface antigen expression (22–29) between avian and mammalian species is extremely high with the result that the analysis of avian T cell development has general relevance to mammalian as well as avian species. One drawback of avian models of T cell development and activation has been a relative paucity of defined transformed cells, compared with mammalian species, as sources of material for the analysis of the biochemistry of cell surface molecules and their interactions.

In this report we demonstrate that transformed T cells can be derived from exposure of normal ex vivo or activated normal chicken T cells to REV(T)(CSV) in vitro. Such T cells express either TCR-α/β or γ/δ, the latter representing the first stable isolation of transformed chicken γ/δ T cells. Whereas REV(T)(CSV) transformation of ex vivo spleen cells generated cell lines containing exclusively B cells, exposure of mitogen-activated spleen cells to REV(T)(CSV) resulted in transformed lines containing predominantly T cells, and including both CD4+ and CD8+ cells. While transformed T cell lines derived from mitogen-activated spleen cells were unstable, lines derived from normal bone marrow could be cloned with high efficiency, and T cell clones of either α/β or γ/δ lineage were generated. Consequently, while the activity of the v-rel oncogene is functionally expressed in T cells, parameters other than v-rel expression limit the immortalization of chicken T cells by REV(T).

Materials and Methods

Cells. Bursa, spleen, bone marrow, and PBL were prepared from 6–8-wk-old SC chickens (Hyline International, Dallas Center, IA) as described elsewhere (29). Spleen T cells were prepared by incubation of normal spleen cells with the anti-Bu-1 antibodies 21-1A4 and Pu5.11G2 (30) for 15 min followed after washing by incubation at 37°C for 30 min with rabbit anti-mouse Ig and guinea pig complement (Cedarlane Laboratories, Hornby, Ontario, Canada), each preabsorbed on chicken lymphoid cells.

Tissue Culture. All tissue culture was performed in IMDM supplemented as described elsewhere (13). Supernatant from the S2A cell line was filtered, aliquotted, stored at −70°C, and thawed immediately as a source of REV(T)(CSV) (13). Spleen cells were activated by culture for 3–4 d at 5 × 10^6 cells/ml in the presence of 3 μg/ml Con A (Sigma Chemical Co., St. Louis, MO), Con A was subsequently removed by washing cells in 0.05 M methyl α-d-mannopyranoside (Sigma Chemical Co.) before further use.

Cells were transformed by culture at 2–5 × 10^6/ml in the presence of 75% S2A supernatant. When cell growth was evident, cells were passaged in IMDM without further addition of virus-containing supernatants. Cell lines were cloned by growth from cell lines derived from mitogen-activated spleen cells to REV(T)(CSV) resulted in transformed lines containing predominantly T cells, and including both CD4+ and CD8+ cells. While transformed T cell lines derived from mitogen-activated spleen cells were unstable, lines derived from normal bone marrow could be cloned with high efficiency, and T cell clones of either α/β or γ/δ lineage were generated. Consequently, while the activity of the v-rel oncogene is functionally expressed in T cells, parameters other than v-rel expression limit the immortalization of chicken T cells by REV(T).

Results

REV(T)(CSV) Transforms Chicken T Cells Expressing Either TCR-α/β or γ/δ. Exposure of chickens in vivo to the REV(T)(CSV) retrovirus leads to the rapid induction of polyclonal B cell lymphomas that can readily be adapted to in vitro growth (11, 12). More recently, however, exposure of cyclophosphamide-treated (B cell–deficient) chickens to REV(T(REVA)) resulted in the induction of T cell tumors in vivo (18). We have shown that transformed cells expressing surface Ig can be isolated and cloned from embryo bone marrow after exposure to REV(T)(CSV) in vitro (13). However, infection of cells from embryo bone marrow with REV(T)(CSV) induced transformation of not only slg+ cells but a substantial population of slg− cells (13) whose lineage is currently unclear but nonetheless demonstrated that slg expression is not a prerequisite for REV(T)(CSV) transformation in vitro.

Exposure of unfractionated lymphocytes from spleen, bursa, or peripheral blood of 3–8-wk-old normal chickens to REV(T)(CSV) in vitro resulted in the generation of transformed cells, essentially all of which expressed slg (Fig. 1). In contrast, exposure of bone marrow cells from 6-wk-old chickens to REV(T)(CSV) resulted in the generation of cell lines that routinely contained considerably <100% slg+ cells. Typically, 2 wk after initiation, one such culture contained >37% of slg+ cells (Fig. 1 d) and was clearly polyclonal as judged by the multiple foci of cell growth in the primary cultures.

Materials and Methods

Cells. Bursa, spleen, bone marrow, and PBL were prepared from 6–8-wk-old SC chickens (Hyline International, Dallas Center, IA) as described elsewhere (29). Spleen T cells were prepared by incubation of normal spleen cells with the anti-Bu-1 antibodies 21-1A4 and Pu5.11G2 (30) for 15 min followed after washing by incubation at 37°C for 30 min with rabbit anti-mouse Ig and guinea pig complement (Cedarlane Laboratories, Hornby, Ontario, Canada), each preabsorbed on chicken lymphoid cells.

Tissue Culture. All tissue culture was performed in IMDM supplemented as described elsewhere (13). Supernatant from the S2A cell line was filtered, aliquotted, stored at −70°C, and thawed immediately as a source of REV(T)(CSV) (13). Spleen cells were activated by culture for 3–4 d at 5 × 10^6 cells/ml in the presence of 3 μg/ml Con A (Sigma Chemical Co., St. Louis, MO), Con A was subsequently removed by washing cells in 0.05 M methyl α-d-mannopyranoside (Sigma Chemical Co.) before further use.

Cells were transformed by culture at 2–5 × 10^6/ml in the presence of 75% S2A supernatant. When cell growth was evident, cells were passaged in IMDM without further addition of virus-containing supernatants. Cell lines were cloned by growth from cell lines derived from mitogen-activated spleen cells to REV(T)(CSV) resulted in transformed lines containing predominantly T cells, and including both CD4+ and CD8+ cells. While transformed T cell lines derived from mitogen-activated spleen cells were unstable, lines derived from normal bone marrow could be cloned with high efficiency, and T cell clones of either α/β or γ/δ lineage were generated. Consequently, while the activity of the v-rel oncogene is functionally expressed in T cells, parameters other than v-rel expression limit the immortalization of chicken T cells by REV(T).

Results

REV(T)(CSV) Transforms Chicken T Cells Expressing Either TCR-α/β or γ/δ. Exposure of chickens in vivo to the REV(T)(CSV) retrovirus leads to the rapid induction of polyclonal B cell lymphomas that can readily be adapted to in vitro growth (11, 12). More recently, however, exposure of cyclophosphamide-treated (B cell–deficient) chickens to REV(T(REVA)) resulted in the induction of T cell tumors in vivo (18). We have shown that transformed cells expressing surface Ig can be isolated and cloned from embryo bone marrow after exposure to REV(T)(CSV) in vitro (13). However, infection of cells from embryo bone marrow with REV(T)(CSV) induced transformation of not only slg+ cells but a substantial population of slg− cells (13) whose lineage is currently unclear but nonetheless demonstrated that slg expression is not a prerequisite for REV(T)(CSV) transformation in vitro.

Exposure of unfractionated lymphocytes from spleen, bursa, or peripheral blood of 3–8-wk-old normal chickens to REV(T)(CSV) in vitro resulted in the generation of transformed cells, essentially all of which expressed slg (Fig. 1). In contrast, exposure of bone marrow cells from 6-wk-old chickens to REV(T)(CSV) resulted in the generation of cell lines that routinely contained considerably <100% slg+ cells. Typically, 2 wk after initiation, one such culture contained >37% of slg+ cells (Fig. 1 d) and was clearly polyclonal as judged by the multiple foci of cell growth in the primary cultures.
Each of the REV-T(CSV)–transformed cell lines described in Fig. 1, including the line from bone marrow, contained exclusively cells expressing high levels of MHC class II (detected with the 21-1A6 mAb [35]) and undetectable levels of the Bu-1 B cell surface antigen (detected with the Fu5.1G2 mAb [30]) (data not shown). This is consistent with the observed phenotype of cells transformed with REV-T(CSV) in vivo (11) or in vitro (13).

Another similarly transformed cell line derived from juvenile bone marrow contained ~35% of slg+ cells 6 wk after transformation and was further analyzed for the expression of T cell surface antigens (Fig. 2). Approximately 45% of cells within this line expressed the CD3 complex as determined by the CT3 mAb (Fig. 2 b). Fewer than 1% of cells expressed the CD4 accessory molecule, whereas 47% of cells expressed CD8 (Fig. 2, c and d).

To estimate the frequency of cells transformed by REV-T(CSV), titrated numbers of bone marrow cells were cultured in soft agar in the presence of virus. The formation of macroscopic colonies was dependent on the presence of REV-T(CSV), and the frequency of bone marrow cells transformed by the virus was ~1 in 500–1,000 (Table 1). Since the majority of cells transformed by REV-T(CSV) were either CD3+ or slg+, and these cells represent ~5% of the ex vivo bone marrow cell suspensions, as judged by flow cytometry, the frequency of bone marrow lymphoid cells transformed can be estimated at ~1 in 20–40.

Chicken T cells, as is the case in mammalian species, can be divided into those expressing TCR-α/β heterodimers and those expressing TCR-γ/δ heterodimers (20). 8 wk after transformation, the bone marrow–derived cell line described in Fig. 2 contained 15% cells expressing TCR-γ/δ as defined by the TCR1 (23) mAb (Fig. 3 a, γ-axis). The chicken TCR

Table 1. Frequency of Bone Marrow Cells Transformed by REV-T(CSV)

| Cell number | REV-T(CSV) | Per plate | Per 10^2 lymphocytes |
|-------------|------------|-----------|----------------------|
| 2 x 10^6    | +          | 3,300     | 3.2                  |
| 2 x 10^6    | +          | 2,600     | 2.6                  |
| 2 x 10^6    | -          | <10       | -                    |
| 6 x 10^5    | +          | 1,600     | 5.4                  |
| 6 x 10^5    | -          | <10       | -                    |
| 2 x 10^4    | +          | 650       | 6.4                  |
| 2 x 10^4    | -          | <10       | -                    |

* Macroscopic colonies were counted after 8–11 d of culture.
† Bone marrow cells were cultured in the presence of 75% S2A3 supernatant in 15-ml soft agar cultures.
§ Colonies per 10^6 lymphocytes were calculated from the number of colonies per plate by accounting for the percentage of lymphocytes in the starting bone marrow cell populations (typically 3–5%).
bone marrow line expressed high levels of the CD8 molecule (38), consistent with the surface CD3-CD8+ phenotype of some cells within the bone marrow line.

Transformation of CD4+ T Cells from Activated Spleen Cell Populations. The transformation of T cells from bone marrow was surprising since juvenile chicken bone marrow typically contains <5% T cells compared with 40–60% T cells in the spleen. We considered the possibility therefore that the relative susceptibility of bone marrow T cells to REV(T(CSV))-mediated transformation was a reflection of differences in T cell physiology compared with splenic T cells. Spleen cells were therefore stimulated for 3 d in vitro with Con A, after which time such populations routinely contained >95% CD3+ cells, ~20% CD4+ cells, ~75% CD8+ cells, and <3% slg+ B cells. Con A-activated spleen cells were then cultured in the presence of REV(T(CSV))-containing supernatants without any further stimulus. Continued and extensive cell growth occurred in cultures containing virus, whereas cultures not containing virus had no viable cells after 48–72 h. As before, transformed cell growth was clearly polyclonal and rapid. Staining this population 12 d after transformation revealed ~10% TCR-γ/δ cells, all of which were CD4- and about half of which were CD8+ (Fig. 4), similar to the phenotypes of TCR-γ/δ+ cells transformed from juvenile chicken bone marrow.

55% of cells within the line expressed TCR-α/β using the Vβ1 gene (TCR2+). Of these, about one-quarter (24%) expressed CD4, and three-quarters (76%) expressed CD8. Similarly, of the 30% of cells expressing TCR-α/β using the Vβ2 gene (TCR3+), 25% expressed CD4, and 75% expressed CD8 (Fig. 4). Double staining revealed that CD4 and CD8 in this line were expressed on mutually exclusive populations of cells (data not shown). Consequently, it is clear that REV(T(CSV)) can transform CD4+ and CD8+ T cells, and that the CD4+ population of cells transformed is restricted to those expressing TCR-α/β.

The frequency of Con A-activated splenic T cells transformed by REV(T(CSV)) was estimated by limiting dilution in 10 μl Terasaki cultures containing 75% S2A3 supernatant. Regression analysis of the limiting dilution (Fig. 5) demonstrated that ~1 in 420 activated splenic T cells was transformed by REV(T(CSV)). As before, growth was completely dependent on the presence of virus.

Activation-induced Receptors for REV(T(CSV)) on T Cells. It...
seemed possible that the failure to efficiently transform ex vivo splenic T cells with REV-T(CSV) might be, at least in part, a consequence of a lack of T cell surface expression of a receptor for the virus. Consistent with this hypothesis, purified splenic T cells (95% CD3+) were unable to absorb the transforming activity from REV-T(CSV)-containing supernatants (Fig. 6), as assayed by the ability of absorbed supernatants to subsequently transform ex vivo bursal cells. In contrast, Con A-activated spleen cells, after washing in 0.05 M methyl α-D-mannopyranoside to remove cell-bound Con A, absorbed transforming activity more efficiently than did ex vivo bursal cells, an established target of REV-T(CSV) transformation. Consequently, activation of splenic T cells induced expression of functional receptors for the REV-T(CSV) retrovirus.

Stability and Cloning of REV-T(CSV)-transformed T Cells. The transformed lines derived from bone marrow were relatively stable over extended periods of time in vitro. Changes in the relative proportion of phenotypically distinct cells were consistent with small differences in growth rates among the different cell populations within the lines. In contrast, transformed lines derived from Con A-activated spleen were not stable. After ~2-3 wk of growth, the frequencies of CD3+, CD4+, and CD8+ cells declined from those seen in Fig. 4 such that by 4-6 wk after transformation the majority of cells within the line were negative for these T cell markers.

To determine whether the T cells transformed from different sources had differing potentials for long-term growth, we established limiting dilution cultures of REV-T(CSV)-trans-
Figure 7. Clonability of REV(T(CSV))-transformed T cells derived from bone marrow or spleen. REV(T(CSV))-transformed cells were plated at a concentration one cell/10 μl in 10-μl cultures in the absence of added filters or virus-containing supernatants. (□) The percent of cultures with cell growth over the first week of culture; (■) the percent of cultures containing cells that maintained growth subsequent to passage into 200 μl followed by 2-ml cultures.

Limiting dilution cultures established from bone marrow showed a high cloning efficiency within the first week of culture (30–45% of wells with growth). The majority of these cultures (>65%) maintained growth subsequent to passage into larger cultures and continued to grow for weeks to months (Fig. 7). Large panels of clones were thereby derived from transformed bone marrow lines, and ~200 clones (those derived from microscopically observed single cells) were examined for cell surface antigen expression. Representative examples of the cloned cell phenotypes are shown in Fig. 8. In general, the relative frequency of clones of a given phenotype corresponded to the proportion of cells with that phenotype in the starting population of cells from which they were cloned. After cloning, the levels of expression of TCR (either α/β or γ/δ) and CD8 were stable for weeks to months thereafter.

In contrast, considerably less short-term growth was observed in limiting dilution cultures established from REV(T(CSV))-transformed Con A-activated spleen cells (Fig. 7). Typically, <15% of the input cells grew over the first week of culture. The proportion of cultures in which continued cell growth was maintained was extremely small (<2%). Among the few cells cloned from these lines, none expressed the T cell markers CD3, CD4, or CD8. This suggests that

Figure 8. Surface antigen expression among REV(T(CSV))-transformed clones. Clones isolated from REV(T(CSV))-transformed bone marrow as described in the text were stained with TCR1, TCR2, TCR3, CT3 (anti-CD3), EP96 (anti-CD4), or EP72 (anti-CD8), each being detected with the appropriate FITC- or PE-conjugated anti-isotype reagent as described. Profiles from 10,000 viable cells are shown.
Clonal populations of chicken T cells transformed by exposure of molecules expressed on the T cell surface. A major use of REV-T in vitro. Such clones obviously provide an ideal source of material for characterization and ultimate cloning of transformed cells has been the definition of the biochem-

**Discussion**

The REV-T(CSV)-transformed clones of chicken T cells derived from bone marrow and expressing either TCR-α/β or -γ/δ heterodimers represent the first demonstration of stable cell growth as being immortalized and distinguish this from T cell transformation, while appreciating that at this stage it is not possible to determine whether they will indeed grow indefinitely. For practical purposes, however, we have observed continued clonal growth for at least 4-6 mo, sufficient time to generate very large quantities of cloned cells.
We can therefore distinguish, based on the growth properties of T cells transformed from bone marrow as compared with activated spleen, T cell transformation from T cell immortalization. Since both T cell transformation in the short term and T cell immortalization require the target cell to express REV-T(CSV) receptors, parameters other than viral receptor expression must limit the long-term growth potential of REV-T(CSV)-transformed cells. Such parameters may also limit the transformation of B cells or ex vivo splenic T cells such that REV-T(CSV) receptor expression is not the only limit to target cell transformation. Consequently, in vitro activation of splenic T cells may lead not only to REV-T(CSV) receptor expression but also to other (intracellular) changes in T cell physiology required for transformation.

The transforming oncogene of REV-T(CSV) is v-rel (3, 4), the founding member of the rel family of cytosolic and/or nuclear proteins, which includes its cellular homologue, c-rel, as well as the NFκB complex proteins p50 and p65 (41-43). The v-rel-encoded protein pp59v-rel has been found in the cytosol, associated with other members of the rel family, notably pp75v-rel (44), as well as a protein of 40-kD, pp40 (45), a member of the avian IκB family (46) and higher molecular mass proteins (47), which include the p105 precursor of NFκB p50 (48). The molecular mechanism by which v-rel expression transforms target cells is currently unclear, but has been linked to its ability to form intracytoplasmic complexes with other proteins (49). There is evidence that v-rel functions as a transcriptional regulator (50, 51), although whether this function is mediated directly by the regulation of transcription by pp59v-rel-containing complexes, or indirectly, by pp59v-rel sequestering normally active transcription regulating complexes, is unclear. Nonetheless, under either circumstance the oncogenic properties of v-rel likely depend on the endogenous expression and/or activation of other members of the rel protein family, possibly including NFκB. Mitogen activation of mammalian T cells leads to the rapid activation of NFκB (52). Therefore, the susceptibility of mitogen-activated chicken T cells to REV-T(CSV)-induced transformation may reflect not only induction of viral receptor expression, but activation of intracellular rel-related signal-transducing complexes.

The dissociation of transformation from immortalization further suggests that the retroviral integration and transcription of v-rel, driven by the retroviral LTR promoter required for transformation, is not sufficient for immortalization. Thus, intracellular factors other than v-rel expression are likely required to maintain the transformed state leading to cell immortalization. The difference in growth properties between bone marrow T cells and mitogen-activated splenic T cells demonstrates that independent of whether or not the bone marrow T cells are activated in vivo, they are nonetheless physiologically distinct from in vitro mitogen-activated splenic T cells. Therefore, it is possible that not only does the initial transformation of cells by v-rel require the coincident presence of active complexes containing rel-related proteins, but that the persistence of the transformed phenotype leading to immortalization requires that such complexes be constitutively active. The failure to isolate transformed and/or immortalized CD4+ T cells from bone marrow, despite their presence in the ex vivo bone marrow population, further suggests that there are physiological differences between CD4+ and CD4- (including but not restricted to CD8+ ) T cells in the normal bone marrow. Whether this reflects differences in retroviral receptor expression or intracellular differences is not currently clear.

Short-term transformation of in vitro activated chicken T cells by exposure to REV-T(REVA) in vitro has been described recently elsewhere (53). While it is difficult to judge from these results which populations of splenic T cells were transformed, growth of REV-T-infected splenic T cells was maintained in supernatants enriched for IL-2. It is quite possible therefore that the growth of the REV-T(CSV)-transformed T cells derived from activated spleen cells described here might be extended in the presence of appropriate cytokines. While this approach does not yield truly immortalized cells, dependent as they are on exogenous cytokines, it might provide a means of cloning and expanding selected populations of chicken T cells, since it is likely that the initial mitogenic activation can be replaced by antigen-specific (or anti-TCR antibody) induced activation.

The efficiency of transformation of bone marrow cells (based on colony formation in soft agar; Table 1) suggests that at least 1 in 50 ex vivo bone marrow T cells can be transformed by REV-T(CSV). About 1 in 400 activated splenic T cells are transformed by REV-T(CSV), as judged by limiting dilution (Fig. 5). These frequencies compare very favorably with human T cell transformation by HTLV-I or HTLV-II, where efficiencies of transformation are considerably lower and frequently require that the target cells be cocultured with virus-producing cell lines (54). To date, rel-based constructs have not been effective in transforming mammalian cells. However, the results demonstrated here suggest that should this limitation be overcome, oncogenic forms of mammalian rel should provide a potent means of transforming mammalian T cells.

We thank Dr. Chen-lo Chen for mAbs, Dr. Eric Humphries for the S2A3 cell line, and Dr. C. Paige for advice on soft agar cultures.

This work was supported the National Cancer Research Institute of Canada and by the Medical Research Council of Canada (MA 10040). M. J. H. Ratcliffe is a Senior Chercheur-boursier of the Fonds de la Recherche en Santé du Québec. T. Benatar is a recipient of a postgraduate studentship from the Cancer Research Society Inc.
References

1. Theilen, G.H., R.F. Ziegel, and M.J. Twiehaus. 1966. Biological studies with RE virus that induces reticuloendotheliosis in turkeys, chickens and Japanese quail. J. Natl. Cancer Inst. 37:731.

2. Chen, I.S.Y., T.W. Mak, J.J. O’Rear, and H.M. Temin. 1981. Characterization of reticuloendotheliosis virus strain T DNA and isolation a novel variant of reticuloendotheliosis virus strain T by molecular cloning. J. Virol. 40:800.

3. Stephens, R.M., N.R. Rice, R.R. Hiebsch, H.R. Bose Jr., and R.V. Gilden. 1983. Nucleotide sequence of v-rel: the oncogene of reticuloendotheliosis virus. Proc. Natl. Acad. Sci. USA. 80:6229.

4. Garson, K., H. Percival, and C.Y. Kang. 1990. The N-terminal env-derived amino acids of v-rel are required for full transforming activity. Virology. 177:106.

5. Hoelzer, J.D., R.B. Franklin, and H.R. Bose. 1979. Transformation by reticuloendotheliosis virus, development of a focus assay and isolation of a nontransforming virus. Virology. 93:20.

6. Mummman, H.C., and M.J. Twiehaus. 1971. Pathogenesis of reticuloendotheliosis virus disease in chicks. An acute running disease. Avian Dis. 15:483.

7. Rup, B.J., J.L. Spence, J.D. Hoelzer, R.B. Lewis, C.R. Carpenter, A.S. Rubin, and H.R. Bose. 1979. Immunosuppression induced by avian reticuloendotheliosis virus: mechanism of induction of the suppressor cell. J. Immunol. 123:1362.

8. Beug, H., H. Muller, S. Grieser, G. Doederlein, and T. Graf. 1981. Hematopoietic cells transformed in vitro by REV strain. Virology. 115:295.

9. Lewis, R.B., J. McClure, B. Rup, W. Niesel, R.F. Garry, J.D. Hoelzer, K. Nazerian, and H.R. Bose. 1981. Avian reticuloendotheliosis virus: identification of the target cell for transformation. Cell. 25:421.

10. Shibuya, T., I. Chen, A. Howatson, and T.W. Mak. 1982. Morphological, immunological and biochemical analysis of chicken spleen cells transformed in vitro by reticuloendotheliosis virus strain T. Cancer Res. 42:2722.

11. Barth, C.F., and E.H. Humphries. 1988. A nonimmunosuppressive helper virus allows high efficiency induction of B cell lymphomas by reticuloendotheliosis virus strain T. J. Exp. Med. 167:89.

12. Barth, C.F., and E.H. Humphries. 1988. Expression of v-rel induces mature B-cell lines that reflect the diversity of avian immunoglobulin heavy- and light-chain rearrangements. Mol. Cell. Biol. 8:5358.

13. Benatar, T., S. Iacampo, L. Tkalec, and M.J.H. Ratcliffe. 1991. Expression of immunoglobulin genes in the avian embryo bone marrow revealed by retroviral transformation. Eur. J. Immunol. 21:2529.

14. Benatar, T., L. Tkalec, and M.J.H. Ratcliffe. 1992. Stochastic rearrangement of immunoglobulin variable region genes in chicken B cell development. Proc. Natl. Acad. Sci. USA. 89:7615.

15. Zhang, J., W. Bargmann, and H.R. Bose, Jr. 1989. Rearrangement and diversification of immunoglobulin light chain genes in lymphoid cells transformed by reticuloendotheliosis virus. Mol. Cell. Biol. 9:4970.

16. Chen, L., M.Y. Lim, H.R. Bose, Jr., and J.M. Bishop. 1988. Rearrangements of chicken immunoglobulin genes in lymphoid cells transformed by the avian retroviral oncogene v-rel. Proc. Natl. Acad. Sci. USA. 85:549.

17. Zhang, J., W. Olson, D. Ewert, W. Bargmann, and H.R. Bose, Jr. 1991. The v-rel oncogene of avian reticuloendotheliosis virus transforms immature and mature lymphoid cells of the B lineage in vitro. Virology. 183:457.

18. Barth, C.F., D.L. Ewert, W.C. Olson, and E.H. Humphries. 1990. Reticuloendotheliosis virus REV(T/REVA)-induced neoplasia: development of tumors within the T lymphoid and myeloid lineages. J. Virol. 64:6054.

19. LeDouarin, N.M., and F.V. Jotereau. 1975. Tracing of cells of the avian thymus through embryonic life in interspecific chimera. J. Exp. Med. 142:17.

20. Chen, C.H., R.P. Bucy, and M.D. Cooper. 1990. T cell differentiation in birds. Semin. Immunol. 2:79.

21. Vainio, O., and O. Lasila. 1989. Chicken T cells: differentiation antigens and cell-cell interactions. CRC Crit. Rev. Virology. 2:97.

22. Chen, C.-L.H., L.L. Ager, G.L. Gartland, and M.D. Cooper. 1986. Identification of a T3/T cell receptor complex on chickens. J. Exp. Med. 164:375.

23. Sowder, J.E., C.H. Chen, L.L. Ager, M.M. Chan, and M.D. Cooper. 1988. A large subpopulation of avian T cells express a homologue of the mammalian Tγ/δ receptor. J. Exp. Med. 167:315.

24. Chen, C.-L.H., J. Cihak, U. Losch, and M.D. Cooper. 1988. Differential expression of two T cell receptors, TCRγ and TCRδ, on chicken lymphocytes. J. Immunol. 140:1853.

25. Chen, C.H., J.T. Sowder, J.M. Lahti, J. Cihak, U. Losch, and M.D. Cooper. 1989. TCRγ: a third T cell receptor in the chicken. Proc. Natl. Acad. Sci. USA. 86:2352.

26. Chan, M.M., C.-L.H. Chen, L.L. Ager, and M.D. Cooper. 1988. Identification of the avian homologues of mammalian CD4 and CD8 antigens. J. Immunol. 140:2133.

27. Veillette, A., and M.J.H. Ratcliffe. 1991. Avian CD4 and CD8 interact with a cellular tyrosine protein kinase homologous to mammalian p56lck. Eur. J. Immunol. 21:397.

27. Vainio, O., B. Riwar, M.H. Brown, and O. Lasila. 1991. Characterization of the putative avian CD2 homologue. J. Immunol. 147:1593.

28. Paramithiotis, E., L. Tkalec, and M.J.H. Ratcliffe. 1991. High levels of CD45 are coexpressed with CD4 and CD8 on avian thymocytes. J. Immunol. 147:3710.

29. Veromaa, T., O. Vainio, E. Eerola, and P. Toivanen. 1988. Monoclonal antibodies against chicken Bu-la and Bu-lb alloantigens. Hybridoma. 7:41.

30. Ratcliffe, M.J.H., and L. Tkalec. 1990. Cross-linking of the surface immunoglobulin on lymphocytes from the bursa of Fabricius results in second messenger generation. Eur. J. Immunol. 20:1073.

31. Lahti, J.M., C.H. Chen, L.W. Tjoelker, J.M. Pickel, K.A. Schat, B.W. Calnek, C.B. Thompson, and M.D. Cooper. 1991.
Two distinct αβ T cell lineages can be distinguished by the differential usage of T cell receptor VB gene segments.  

33. Yelton, D.E., C. Desaymard, and M.D. Scharf. 1981. Use of monoclonal anti-mouse immunoglobulins to detect mouse antibody. Hybridoma. 1:5.

34. Wilhelmsen, K.C., and H.M. Temin. 1984. Structure and dimorphism of c-rel (turkey), the cellular homologue to the oncogene of reticuloendotheliosis virus strain T. J. Virol. 49:521.

35. Veromaa, T., O. Vainio, S. Jalkanen, E. Eerola, K. Granfors, and P. Toivanen. 1988. Expression of B-L and Bu-1 antigen in chickens bursectomized at 60h of incubation. Eur. J. Immunol. 18:225.

36. Tjoelker, L.W., L.M. Carlson, K. Lee, J. Lahti, W.T. McMack, J.M. Leiden, C.H. Chen, M.D. Cooper, and C.B. Thompson. 1990. Evolutionary conservation of antigen recognition: the chicken T-cell receptor β chain. Proc. Natl. Acad. Sci. USA. 87:7856.

37. Bucy, R.P., C.H. Chen, and M.D. Cooper. 1990. Development of cytoplasmic CD3 + T cell receptor-negative cells in the peripheral lymphoid tissues of chickens. Eur. J. Immunol. 20:1345.

38. Bucy, R.P., M. Coltey, C.H. Chen, D. Char, N.M. Le Douarin, and M.D. Cooper. 1989. Cytoplasmic CD3 + surface CD8 + lymphocytes develop as a thymus-independent lineage in chick-quail chimeras. Eur. J. Immunol. 19:1449.

39. Ratcliffe, M.J.H., K.M. Coggeshall, M.K. Newell, and M.H. Julius. 1992. T cell receptor aggregation, but not dimerization, induces increased cytosolic calcium concentrations and reveals a lack of stable association between CD4 and the T cell receptor. J. Immunol. 148:1643.

40. Vainio, O., T. Veromaa, E. Eerola, P. Toivanen, and M.J.H. Ratcliffe. 1988. Antigen-presenting cell-T cell interaction in the chicken is MHC class II restricted. J. Immunol. 140:2864.

41. Wilhelmsen, K.C., K. Eggleton, and H.M. Temin. 1984. Nuclear acid sequences of the oncogene v-rel in reticuloendotheliosis virus strain T and its cellular homologue, the proto-oncogene c-rel. J. Virol. 52:172.

42. Ghosh, S., A.M. Gifford, L.R. Riviere, P. Tempst, G.P. Nolan, and D. Baltimore. 1990. Cloning of the p50 DNA-binding subunit of NFkB: homology to rel and dorsal. Cell. 62:1019.

43. Nolan, G.P., S. Ghosh, H.C. Liou, P. Tempst, and D. Baltimore. 1991. DNA binding and IxB inhibition of the cloned p65 subunit of NFkB, a rel-related polypeptide. Cell. 64:961.

44. Lim, M.Y., N. Davis, J. Zhang, and H.R. Bose Jr. 1990. The v-rel oncogene product is complexed with cellular proteins including its proto-oncogene product and heat shock protein 70. Virology. 175:149.

45. Tung, H.Y.L., W.J. Bargmann, M.Y. Lim, and H.R. Bose, Jr. 1988. The v-rel oncogene product is complexed to a 40kD phosphoprotein in transformed lymphoid cells. Proc. Natl. Acad. Sci. USA. 85:6299.

46. Davis, N., S. Ghosh, D.L. Simmons, P. Tempst, H.-C. Liou, D. Baltimore, and H.R. Bose, Jr. 1991. Rel associated p40: an inhibitor of the rel family of transcription factors. Science (Wash. DC). 253:1268.

47. Simek, S., and N.R. Rice. 1988. p59~rel, the transforming protein of reticuloendotheliosis virus, is complexed with at least four other proteins in transformed chicken lymphoid cells. J. Virol. 62:4730.

48. Capobianco, A.J., D. Chang, G. Mosialos, and T.D. Gilmore. 1992. p50, the NFkB p50 precursor protein, is one of the cellular proteins complexed with the v-rel oncoprotein in transformed chicken spleen cells. J. Virol. 66:3758.

49. Garson, K., and C.-Y. Kang. 1990. Mapping of the functional domains of the v-rel oncogene. Oncogene. 5:1431.

50. Gelas, C., and H.M. Temin. 1988. The v-rel oncogene encodes a cell specific transcriptional activator of certain promoters. Oncogene. 3:349.

51. Hannink, M., and H.M. Temin. 1989. Transactivation of gene expression by nuclear and cytoplasmic rel proteins. Mol. Cell. Biol. 9:4323.

52. Bohnlein, E., J.W. Lowenthal, M. Siekevitz, D.W. Ballard, B.R. Franz, and W.C. Greene. 1988. The same inducible nuclear proteins regulate mitogen activation of both the interleukin-2 receptor-alpha gene and type 1 HIV. Cell. 53:827.

53. Schat, K.A., W.D. Pratt, R. Morgan, D. Weinstock, and B.W. Calnek. 1992. Stable transfection of reticuloendotheliosis virus transformed lymphoblastoid cell lines. Avian Dis. 36:432.

54. Yamamoto, N., M. Okada, Y. Koyanagi, Y. Kannagi, M. Kannagi, and Y. Hinuma. 1982. Transformation of human leukocytes by cocultivation with an adult T cell leukemia virus producer cell line. Science (Wash. DC). 217:737.