Retroviral Transformation In Vitro of Chicken T Cells Expressing Either $\alpha/\beta$ or $\gamma/\delta$ T Cell Receptors by Reticuloendotheliosis Virus Strain T

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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 $\alpha/\beta$ or $\gamma/\delta$ T cell receptors (TCRs) in proportions roughly equivalent to the proportions of TCR-$\alpha/\beta$ and TCR-$\gamma/\delta$ T cells found in the normal bone marrow in vivo. Essentially all TCR-$\alpha/\beta$-expressing transformed bone marrow-derived T cells expressed CD8, whereas few, if any, expressed CD4. In contrast, among TCR-$\gamma/\delta$ 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-T(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-$\alpha/\beta$. 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-T(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-T(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-T 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-T(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 S3A cell line was filtered, aliquotted, and stored at −70°C, and thawed immediately before use as a source of REV(T(CSV)) (13). Spleen cells were activated by culture for 3–4 d at 5 × 105 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 × 109/ml in the presence of 75% S3A 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 microscopically observed single cells in 10-μl Terrasaki cultures established in the absence of any exogenous filler cells, as described elsewhere (13).

The frequency of bone marrow cells transformed by REV(T(CSV)) was established by colony formation in 15 ml 0.3% Bacto-Agar (Difco Laboratories, Detroit, MI) in IMDM in 8.5-cm-diameter petri dishes containing between 2 × 104 and 1 × 105 cells in the presence of 75% S3A supernatant. Transformation efficiency was measured by quantitation of colonies visible to the naked eye after 8–11 d of culture. The frequency of splenic T cells transformed by REV(T(CSV)) was established by limiting dilution analysis in 10-μl Terrasaki cultures containing titrated numbers of splenic T cells and 75% S3A supernatant. Transformation was defined by growth in 10-μl cultures requiring passage to 100-μl cultures within 7 d.

**Antibodies and Flow Cytometric Analysis.** The mAbs used in these studies were as follows: 6E1, anti–chicken Ig light chain (31); EP96, anti–chicken CD4 (29); EP72, anti–chicken CD8 (29); CT3, anti–chicken CD3 (22); TCR1, anti–chicken TCR-γ/δ (23); TCR2, anti–chicken TCR-α/β (24), defining those T cells using the Vβ1 gene (32); and TCR3, anti–chicken TCR-α/β (25), defining those T cells using the Vβ2 gene (32). Binding of primary antibodies was detected using either FITC-conjugated monoclonal rat anti-mouse Ig (Southern Biotechnology Associates, Birmingham, AL) or FITC- or R-PE-conjugated goat anti-mouse Ig isotype antibodies (Southern Biotechnology Associates, Birmingham, AL), each gating on forward and side scatter.

**Southern Blot Analysis.** Cellular DNA was prepared from ~4 × 106 cells/tracks and digested with a 10-fold excess of restriction enzymes according to manufacturer’s instructions (Pharmacia, Baie D’Urfé, Quebec, Canada). Digested DNA was run on 0.8% agarose gels before depurination, denaturation, blotting onto nylon membranes, and UV crosslinking. Blots were probed with a nick-translated probe comprising a 967-bp EcoRI fragment of the PKW101 plasmid, which hybridizes to v-rel and α- and c-rel sequences (34), incorporating α-[32P]dCTP (ICN Biomedicals Canada, Ltd., Toronto, Ontario, Canada) to a specific activity of between 6 × 108 and 2 × 107 cpm/μg DNA as described elsewhere (13).

**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(REV-A)) 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 sIg- cells but a substantial population of sIg- cells (13) whose lineage is currently unclear but nonetheless demonstrated that sIg expression is not a prerequisite for REV(T(CSV)) transformation in vitro.

Exposure of unfractonated 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 sIg (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% sIg+ cells. Typically, 2 wk after initiation, one such culture contained ~37% of sIg+ 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 Fu5.11G2 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>
<thead>
<tr>
<th>Cell number</th>
<th>REV-T(CSV)</th>
<th>Per plate</th>
<th>Per 10^2 lymphocytes</th>
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<tbody>
<tr>
<td>2 x 10^6</td>
<td>+</td>
<td>3,300</td>
<td>3.2</td>
</tr>
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<td>−</td>
<td>&lt;10</td>
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<tr>
<td>6 x 10^4</td>
<td>+</td>
<td>1,600</td>
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<tr>
<td>2 x 10^3</td>
<td>+</td>
<td>650</td>
<td>6.4</td>
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<td>2 x 10^3</td>
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* 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 10^5 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%).
The transformation of CD3⁻/CD8⁺ cells. Since the proportion of CD8⁺ cells (68% within this transformed bone marrow line at 8 wk after transformation exceeded the number of cells staining with a combination of the three TCR mAbs (a total of 45%; Fig. 3, a–c) or with the CT3 anti-CD3 antibody (data not shown), and further, that some γ/δ T cells were CD8⁺, it seemed probable that the line contained CD8⁺ cells that did not express surface TCR. This was demonstrated directly in Fig. 3 d, where 29% of cells within the line were TCR⁺, CD8⁺. It is possible that these cells represent the transformed counterparts of the TCR0 cells found in the normal chicken that have been characterized as surface TCR⁻, CD3⁻ but contain CD3 component(s) cytoplasmically, as detected by staining with the CT3 antibody on fixed cells (37). At least some TCR0 cells express 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 Terrasaki cultures containing 75% SxA3 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. In
Figure 4. REV(T(CSV)) transformation of CD4+ and CD8+ cells from activated spleen. Transformed cells derived by exposure of activated spleen cells to REV(T(CSV)) were cultured for 14 d and stained for the expression of TCR, CD4, and CD8. Cells were stained with either TCR1 (anti-TCR-γ/δ), TCR2 (anti-Vβ1 TCR), or TCR3 (anti-Vβ2 TCR), each being detected using PE-conjugated anti-β1, in conjunction with either EP96 (anti-CD4) or EP72 (anti-CD8), which were detected with FITC-conjugated anti-μ or FITC-conjugated anti-γ2b, respectively. Contour plots from 20,000 viable cells are displayed, and the numbers in each panel represent the percentage of cells falling within each quadrant.

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-
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 7](image1)

**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.

![Figure 8](image2)

**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 antiisotype reagent as described. Profiles from 10,000 viable cells are shown.

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clonal populations of chicken T cells transformed by exposure to 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 derived from bone marrow and expressing either TCK-α/β or TCR-γ/δ heterodimers represent the first demonstration of stable transformation by the REV-T virus.

The REV-T(CSV) receptor is currently undefined. Increasing numbers of bursal cells or activated T cells absorbed increased amounts of transforming activity from REV-T(CSV)-containing supernatants. This, taken together with the observation that ex vivo splenic T cells fail to absorb such transforming activity, demonstrates that transformation of chicken T cells with REV-T(CSV) requires virus adsorption to cell-specific and saturable receptor(s). REV-T(CSV) transforms both B and T cells in vivo (11, 29) and in vitro. There is no reason to postulate a priori that distinct receptors are used for viral adsorption to B and T cells, and so candidate molecules for the REV-T(CSV) receptor would therefore be expressed on the surface of at least subpopulations of B cells and activated T cells.

The expression of REV-T(CSV) receptors on the surface of ex vivo bone marrow T cells has not been determined directly due to the low numbers of T cells (~2%) in bone marrow suspensions. Consequently, while it is attractive to suggest that at least some bone marrow T cells in vivo express REV-T(CSV) receptors, it cannot formally be ruled out that culture of bone marrow T cells together with other leukocytes and/or stromal cells within the bone marrow population induced viral receptor expression. Such induction would by necessity need to be extremely rapid since the half-life of active REV-T(CSV) in tissue culture is ~4 h.

We have been unable to demonstrate REV-T(CSV)-mediated in vitro transformation of immediately ex vivo splenic T cells from normal chickens. However, exposure of ex vivo splenic cells from surgically bursectomized (B cell-deficient) chickens to REV-T(CSV) in vitro resulted in transformation of T cells with a distribution of phenotypes indistinguishable from those seen in the transformed bone marrow cultures shown in Fig. 3. This is analogous to the transformation of T cells in vivo with REV-T(REV-A) (18) and suggests the presence in normal spleen of a low frequency of T cells susceptible to REV-T transformation.

While T cells transformed from normal bone marrow grew for extended periods of time in vitro in the absence of any exogenous stimulus, T cells transformed from activated spleen cell populations grew for a maximum of 6 wk, typically for 3–5 wk, after transformation and could not be cloned. For the sake of this discussion we will refer to those bone marrow–derived T cells in which we have observed prolonged 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 NFkb 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 pp75c-rel (44), as well as a protein of 40-kD, pp40 (45), a member of the avian Ikb family (46) and higher molecular mass proteins (47), which include the p105 precursor of NFkB 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 NFkb. Mitogen activation of mammalian T cells leads to the rapid activation of NFkb (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(RE-V)) 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 mitogen 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.

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