Haematology and Blood Transfusion Vol. 28
Modern Trends in Human Leukemia V
Edited by Neth, Gallo, Greaves, Moore, Winkler

Contrasuppression, Class I Antigens, and Cancer Immunity*

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When immunologic homeostasis is perturbed by antigen, the failure to accomodate that antigen as "self" usually results in what we observe as an immune response. In the case of tumors, however, a massive bombardment of the system with tumor antigens can induce a potent general suppression of immunity such that the tumor must often be removed before immunity can be demonstrated [1]. Such observations suggest that while there may be antigenic determinants on tumor cells which can serve as targets of immunity, immunoregulatory modification might be necessary for such immunity to become manifest. Thus, immune suppression induced by tumor challenge is probably the major stumbling block to effective immunity against many tumors. In terms of therapy, the activity of the suppressor circuit might serve as a target of effective immunengineering.

Alternatively, we can envision situations in which tumors of the lymphoid system may come under suppressor cell control and thus be rendered benign. For example, Rohrer and Lynch [2] have demonstrated control by suppressor T cells of MOPC-315 myeloma clone growth and secretion. Similar effects have been obtained by Abbas et al. [3]. Suppressor T cells appear in normal people infected with Epstein-Barr virus (EBV) [4], and such T cells have been shown to be capable of inhibiting in vitro transformation of B cells by EBV [5]. In some cases, therefore, *failure* to effect suppression of a proliferating cell may be a cause of cancer, so that therapy must then be aimed at enhancing suppressor cell activity. Such tumors, while rare, may be important for our development of effective tumor therapy.

The ability to up or down regulate immune responses is likely to be a key factor in cancer therapy. While the role of suppressor cells in cancer is an active area of research, little is known about the role of the cells that mediate contrasuppression. Contrasuppression is an immunoregulatory T cell activity which is defined functionally as the ability to interfere with suppressor cell signals. Contrasuppressor effector cells have been shown to express a unique profile of cell surface antigens and to function, at least in part, by rendering helper T cells resistant to suppressor cell signals. Clearly, such an activity could have major consequences for our understanding and control of cancer.

In this brief paper we will discuss the evidence that contrasuppressor T cells have an active role in the immune response to cancer. This will lead us into a hypothetical consideration of the role of class I antigens in the activation of regulatory T cells and the consequences of this theory for immunomodulation and therapy. Finally we will review evidence for the possibility that in some cases, involving transformed cells of the immune system, this regulatory activity might enhance tumor incidence by interfering with the suppressor cells capable of controlling tumor growth.

A. Immune Sequelae to the Activation of Contrasuppression

Relatively little is known about the nature of the signals which initiate contrasup-

* D. R. G. is supported by NIH Training Grant AI 07019

pression, but the dose of immunizing antigen is certainly a key factor. Contrasuppression seems to be induced at doses of antigen optimal for immune responses ([6, 7], T. Lehner, personal communication). Certain antigen-presenting cells, such as Langerhans cells, dendritic cells, and peritoneal exudate macrophages induced by complete (but not incomplete) Freund's adjuvant, preferentially activate the cells of this circuit [8, 9]. Other factors involved in activation have been reviewed elsewhere [10, 11].

Following activation, several events have been elucidated. A circuit of T cell interactions has been defined on the basis of surface characteristics of the communicating T cells and the nature of their functional molecular products.

The first subset that has been characterized as follwing activation is an I-J$^+$, Ly-2 T cell which functions to induce contrasuppression [12]. The I-J determinants detected on the cells and molecules of the contrasuppressor circuit are serologically distinct from those expressed by cells and molecules of the "feedback" suppressor circuit [13]. The product of the inducer cell is a molecule(s) which bears an I-J subregion encoded product and can be absorbed on the immunizing antigen (or closely related antigens). The cross-reactive nature of this antigen recognition distinguishes this molecule from suppressor factors [14], and is potentially extremely important. This will be discussed further in the next section. The contrasuppressor inducer factor must interact with a contrasuppressor transducer cell in order to have its effects. This transducer cell is an I-J$^+$, Ly-1, 2 T cell [12, 14]. The evidence at hand suggests that this interaction is restricted by genes linked to the V region of the Ig locus.

The effector cell of the contrasuppressor circuit is an I-J$^+$, Ly-1 T cell which can be positively selected by adherence to the *Vicia villosa* lectin [15], which distinguishes it from helper cells. Further, its activity can be blocked by the presence of *N*-acetyl-D-galactosamine [16]. This cell functions to render helper T cells (and probably other cells of the immune system) resistant to suppressor cell signals [15].

Further, this cell has the ability to block tolerogenic signals in vivo (allowing immunity to become manifest) [17].

Contrasuppression has been implicated in the generation and transfer of contact sensitivity [17–19], resistance to malaria infections ([10], R. Mogil, personal communication), and development of the hyperimmune state [20, 21]. We consider next the possibility that this activity functions in the immune response to cancer.

B. Evidence for Contrasuppression in Tumor Immunity

While contrasuppressor cells have not been used to modulate directly the immune response in cancer, they have been implicated in a number of systems. In this section we will discuss the involvement of contrasuppression in tumor immunity.

Hamaoka et al. [22] described an immunization protocol which produced hapten-reactive T-lymphocytes in the "absence" of suppressor cells. Recently, Rozyka et al. (manuscript in preparation) have demostrated the production of a potent contrasuppressor factor from cells that were primed using Hamaoka's immunization protocol. Hamaoka et al. [23] have further demonstrated that primed animals can produce effective immunity to haptenated tumor cells. Thus, it is likely that activation of contrasuppression to interfere with suppressor cell activity is responsible for the enhanced immune response against the haptenated tumor cells. This is further supported by the observation that the immunity, with time, became cross reactive, such that after priming resistance could be demonstrated for the same tumor cells without hapten [23]. This may be a reflection of the cross-reactive nature of the contrasuppressor inducer cell discussed above [14], that is the reactions against the hapten-modified tumor-associated antigens raised contrasuppressor cells that protected the cells reacting to "unmodified" antigen from host suppressor mechanisms.

Contrasuppression may be implicated in natural resistance to AKR leukemia virus. Mureullo and McDevitt [24] demonstrated

that the transfer of resistance to oncogenesis was dependent upon an I-J$^+$, Ly-1 T cell, a cell with a "contrasuppressive phenotype". In addition, resistant animals could be rendered sensitive by injecting anti-Ly-1 or *anti-I-J antisera* in vivo. Since the effector cell of contrasuppression is an I-J$^+$, Ly-1 T cell [15], removal of this cell could account for the above observations.

Cells which interfere with suppressor cell function were implicated in genetic resistance to Friend leukemia virus (FLV) by Kumar and Bennett [25]. Susceptibility to leukemogenesis correlates with susceptibility to immunodepression by FLV [26]. Susceptibility to immunodepression was further correlated with ability to induce suppressor cells in vitro with FLV [27]. Resistance to suppressor cell induction by FLV was shown to be effected by a marrow dependent cell ("M cell"). Removal of the M cell allowed induction of suppressor cells in resistant strains [25]. (Contrasuppressor cells have been identified in bone marrow and shown to be involved in regulation of hematopoeisis [28].) Kumar and Bennett went on to describe a "suppressor interfering cell" in the FLV system [29]. This will be considered in more detail in the next section. These observations support a role for contrasuppression in control of immunity to leukemia.

Antibodies to certain tumor antigens may react with immunoregulatory cells [30]. Antisera against the Meth A fibrosarcoma raised in Fl animals, but not syngeneic homozygous animals, have been shown to disrupt contrasuppressor activity. Production of these disruptive antibodies correlates with an increased incidence of metastasis in Fl animals over the parental strain [31].

While it remains to be proven that contrasuppressor cells are needed for optimum tumor immunity, the evidence is compelling that this investigative avenue is worth following. In the next section we will consider the activation of this circuit and hypothesize a role for antigen presentation in the context of class I (rather than class II) antigens.

C. Class I Antigens in Contrasuppression and Tumor Immunity

In recent years it has become dogma that helper T cells recognize antigen in the context of class II surface antigens for the initiation of immune responses. Class I antigens are generally viewed as targets for effector cell (CTL) function, such as in T cell killing of transformed or virally infected targets. With few exceptions Ir gene effects mapping to class I loci mediate responses to viral antigens [32] or minor histocompatibility antigens [33]. It is becoming increasing clear, however, that class I antigen presentation in cell-mediated immunity may well involve activation of immunoregulatory subsets. Such regulation has implications for humoral immunity as well.

Using H-2D region mutants, Stukart et al. [34] demonstrated a role for the H-2D halotype in regulating responses to Moloney leukemia virus, even when the effector cells were directed only at virus associated with K-end antigens. H-2D region control of immune responses has also been observed for radiation leukemia virus-induced tumorigenesis [35], Friend virus-induced splenomegaly [36], T-lymphocyte proliferative autoimmune responses to thyroglobulin [37], antibody levels and cellular infiltration in autoimmune thyroiditis [38], and ability to induce suppression for contact sensitivity with DNFB [39]. Antibody responses to equine myoglobin are regulated by complementing genes in H-2D and I-A [40].

Murine resistance to malaria may depend upon activation of contrasuppressor cells to overcome suppression ([10], R. Mogil, personal communication). Vaccination against fatal malaria infection is dependent upon the transfer of infected reticulocytes which display elevated levels of class I antigens [41]. Resistance, however, does not necessarily depend upon the parasite residing within reticulocytes, as immunization with the organism in reticulocytes leads to protective immunity against a fatal strain that proliferates only in mature red blood cells. This indicates that malarial parasites in reticulocytes are not simply better targets of effector cell activity.

Class I antigens have been shown to be important in induction of immunity in several tumor systems. SJL reticulosarcoma lines bearing H-2D antigens are capable of inducing immunity to lines which lack H-2D [42]. Examination of progressor and regressor lines of a UV-induced sarcoma revealed an anitgenic difference mapping to the H-2D region of the MHC. Again, like the Hamaoka story and the immunity to malaria, the regressor line was found to be capable of inducing immunity to the progressor line which lacks the H-2D linked antigen [43].

In the FLV system, Kumar and Bennett [29] examined an FLV-induced "suppressor interfering cell" which was activated in vitro by genetic mismatch of this cell with its target. (The H-2 haplotype of the FLV-induced suppressor cell was irrelevant.) This allogeneic activation was mapped to H-2D [44].

Recently, a system has been developed to analyze the activaton of contrasuppression by antigen-presenting cell subsets in vitro. Preliminary results suggest that this antigen-specific activation can be blocked by anti-class I (especially H-2D) but not by anti-class II antibodies (in preparation).

In light of the above observations, we propose that antigen presentation in the context of class I antigens, especially H-2D, may be important in initiation of contrasuppression. It may be relevant that dendritic cells, which can activate contrasuppression which leads to *dominant* immunity in vitro [9], are high in H-2D antigen expression [45].

If so, then a strategy for optimal tumor immunity may be elevation of class I antigen expression on the tumor cells to activate contrasuppression and allow dominant immune responsiveness over tumor-induced suppression. Experiments are in progress to test this notion.

D. Flip Side: Contrasuppression in Enhancement of Lymphoid Tumor Development

It is well established that persistent activation of target cells by their hormones can result in transformation and carcinogenesis. Regulatory factors are essentially the hormones of the immune system, and we can propose that persistent activation of their targets can result in neoplasia. Signals which inhibit activation, such as suppressor cell factors, might then serve to prevent lymphoid transformation, whereas activities like contrasuppression might, in some instances, enhance lymphoid tumorogenesis.

For example, Houghton et al. [46] have described a situation in which antigenic hyperimmunization causes the appearance of tumors of cells of the immune system. The fact that several B-cell lymphomas produced in this way react with the immunizing antigen suggests direct involvement of the hyperimmunization protocol. Hyperimmunized animals have been shown to possess a potent antigen-specific contrasuppressive activity [20, 21].

As mentioned above, malaria infections in mice produce a potent contrasuppression coincident with recovery. Such infections can enhance oncogenesis by virus [47]. Whether there is any correlation of these effects is unknown, but suggests an exciting possibility. People infectd with Epstein-Barr virus (EBV) exhibit potent suppressor T-cell activity [4], and such cells have been shown to be capable of inhibiting EBV transformation in vitro [5]. Chronic infection with malaria, however, might induce a general contrasuppression which would interfere with this beneficial immunosuppression to allow expansion of the virus-transformed cells. This is a possible rationale for the association of EBV-induced lymphomas in malarial regions [48].

The MRL mouse is a murine model of systemic lupus erythematosis and lymphoproliferation in which autoimmunity proceeds in the face of general suppression [49]. These animals have been shown to be resistant to tolerance induction [50] and suppressor cell signals [51], probably as a result of excessive contrasuppressor activity [51]. The proliferating cells in these animals have a controlled neoplastic tendency, as suggested by the spontaneous appearance of transformed, tumorgenic lines when these cells are cloned (C. Reinisch, personal communication). An understanding of the role of immunoregulatory T cells in the control of such lymphoid tumors will great-

ly increase our knowledge of lymphocyte regulation and the regulation of transformed cells in general.

E. Conclusion

In this brief discussion, we have outlined our argument that contrasuppression might play an important role in the immune response to cancer. While antigen load often induces active suppression to most tumors, induction of contrasuppression early in the response might allow protective immunity to become dominant. There is suggestive evidence that contrasuppression can be initiated by presentation of antigen in the context of class I antigens, in which case these will have a profound role in determining the outcome (positive versus negative immunity) of a tumor challenge.

Many tumors can potentially be controlled by immune responses *against* the tumor. Certain tumors, in addition, might be affected by the regulatory molecules of the immune system themselves, especially if the tumors are lymphoid in origin. Such tumors might behave anomalously (on the surface), being enhanced by positive influences on immune function and controlled by suppressive signals.

Nevertheless, it is clear that as our understanding of immunoregulation increases, we simultaneously improve our potential for controlling the immune response to cancer and increase our abilites to produce effective therapy.

Acknowledgments

The authors wish to thank Rona Mogil for critical discussion of this manuscript and Astrid Swanson and June Goldstein for expert secretarial assistance.

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