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REVIEW ARTICLES

How do senescing leaves lose photosynthetic activity?

Anil Grover

Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi 110 021, India

Delay in leaf senescence to a maximal extent is an agronomically desired trait. The photosynthetic capacity of senescing leaves is usually much lower than their normal counterparts. The onset of senescence involves major changes in various characteristics of ribulose-1,5-bisphosphate carboxylase/oxygenase, the enzyme which is thought to rate-limit photosynthesis. In addition, certain selective and specific changes take place in the electron transport process of the senescing chloroplasts. This article provides information on senescence-associated alterations in various partial reactions of photosynthesis and on gene expression which accompany this phenomenon.

LEAF senescence is a critical developmental shift in the life of a green plant. The research on leaf senescence has been carried out with wide range of interests. It is a challenging area for plant biologists who wish to understand the fundamental aspects of plant development. The physiologists and biochemists are interested to investigate the metabolic interactions which characterize senescing leaves. To an agronomist, senescence is important because it limits the supply of assimilates to the growing seeds. This phenomenon provides an opportunity to a molecular biologist to unravel mechanism(s) responsible for differential gene expression and poses challenges for devising novel strategies to delay it. The objective of this review is to assess various possible reasons which together result in reduction of photosynthetic capacity of the senescing leaves. It will cover information on interaction of leaf senescence and photosynthesis and give a detailed account of the influence of senescence on ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBP carboxylase) and on photosynthetic light reaction components. Finally, comments on gene expression changes during senescence will be documented. For more details concerning metabolic and regulatory events of leaf senescence,

including those on fundamental aspects of photosynthesis, the reader may refer other relevant reviews[1-6]

Leaf senescence and photosynthesis

As senescence cuts short the active life span, its onset obviously hampers the supply of assimilates from the leaves (source) to the grains (sink). The study of senescence under natural conditions as well as under conditions of constrained environment which often accentuates this phenomenon[7] has, therefore, attracted attention. The physiological studies undertaken in this context have established that leaf senescence is not a phenomenon of deteriorative events. Rather, it is useful in the sense that some of the essential metabolites are moved out of the older leaves for the support of actively growing organs of the plant body[8]. This fact has been particularly emphasized for the remobilization of nitrogen[8,9]. In view of this, current interest aims at delaying onset of leaf senescence (so that more carbon can be harvested) as well as to eventually ensure its completion (to ensure maximum tapping of nitrogen). The objective of delaying senescence is somewhat akin to the idea of having delayed ripening in case of fruits such as tomatoes, so that their shelf life is increased. The genetic engineering experiments have made it possible to delay the ripening process of tomatoes by employing antisense gene of polygalacturonase[10] and 1-aminocyclopropane-1-carboxylate synthase[11]. The possibility of altering plant morphology and leaf senescence has recently been demonstrated by enhancing levels of endogenous cytokinins, achieved through expression of tmr gene of *Agrobacterium tumefaciens* (which encodes the enzyme isopentyl transferase) in tobacco[12]. Further efforts with a similar objective in case of leaf senescence warrant more detailed understanding of the intricacies involved in terms of definite information on enzymatic changes.

The senescing leaves show pronounced changes in the ultrastructure of chloroplast and reduction of their net photosynthetic rate compared to that of normal green leaves[13-17]. Detailed analysis on how this happens is expected to provide information on the early reactions of leaf senescence as well as cascade of events underlying the reasons for the decline of photosynthetic activity.

Metabolism of RuBP carboxylase in senescing leaves

The enzyme RuBP carboxylase constitutes nearly 50 per cent of the leaf soluble protein fraction (usually in the range of 6 to 13 mg per g fresh weight of the leaf tissue) and mediates the key reactions of the Calvin and the glycolate cycles[18]. Among nearly 10 to 20 thousand polypeptides present in the cell of a normal, healthy plant leaf, senescence-associated loss of RuBP carboxylase appears both predominant and preferential. For instance, Williams and Kennedy[19] compared activities of several cellular enzymes at young, mature and senescent stages of leaf development in *Zea mays* and found that the loss of RuBP carboxylase activity is preferential during senescence. In senescing wheat seedlings, it has been reported that the loss of RuBP carboxylase, quantitated immunologically, accounted for 80% of the total loss of soluble proteins[20]. A pronounced decline in the activity of this enzyme has also been noticed in several other plant species (see Table 1 for a list of selected papers as well as for other pertinent comments).

The nitrogen loss and loss of RuBP carboxylase activity from the leaves have been marked to be temporally correlated in several instances (see Table 1). It, therefore, appears that the nitrogen remobilization from leaves to the growing seeds possibly involves metabolism of RuBP carboxylase. Considering the preponderance of this protein in a leaf, this fact can explain why the loss of activity of RuBP carboxylase is predominant (but does not explain why it needs to be preferential!). The assessment of the magnitude of free amino-nitrogen released from the proteolysis of RuBP carboxylase and its contribution towards mobilization of nitrogen to the growing seeds has not been quantified as yet.

Several studies have pointed out that the loss of RuBP carboxylase is a key event leading to the decline in photosynthetic capacity as well as functional autonomy of leaves (see Table 1). Thus evaluating leaf senescence on the basis of RuBP carboxylase could be a useful criterion. Increased activities of proteases have been marked in senescing leaves by numerous workers[21,22]. It has been suggested that the preferential hydrolysis of RuBP carboxylase could possibly involve specific proteolytic enzymes (see Table 1). In order to show specificity, one needs to assay proteolytic activity employing RuBP carboxylase rather than bovine serum albumin or casein as substrate. Two endoproteinases have been partially purified from senescing barley leaves which could effectively hydrolyse RuBP carboxylase[23]. An acid proteinase which could hydrolyse RuBP carboxylase has also been partially purified from wheat leaves[24,25]. These experiments prove that the RuBP carboxylase protein is degraded by specific proteinases but do not rule out the fact whether the same proteinases affect other cellular proteins or not. In case the RuBP carboxylase-degrading proteinases are 'dedicated' in their action, it might be possible to delay the loss of RuBP carboxylase by employing antisense technology. Since RuBP carboxylase is a chloroplastic

Table 1. Selected reports on leaf-senescence associated changes in characteristics of RuBP carboxylase enzyme The listing has been done chronologically for each of the three different aspects of RuBP carboxylase

Species	Comments	Ref.
RuBP carboxylase activity		
Perilla frutescens	RuBP carboxylase activity reached its maximum prior to leaf expansion and was followed by a rapid decline.	35
Hordeum vulgare	Most of the total soluble protein loss was accounted for by the decrease in RuBP carboxylase protein. Kinetin, light and cycloheximide prevented the loss of RuBP carboxylase protein to a large extent.	75
Triticum aestivum	Decrease in RuBP carboxylase activity was greater than the decrease of net photosynthesis in the early part of senescence. During the time of grain-filling, specific activity, rather than the enzyme protein *per se*, declined.	76
Triticum aestivum	Change in relative rates of photosynthesis and photorespiration did not reflect a change in RuBP carboxylase to oxygenase *in vitro*.	77
Triticum aestivum	Preferential loss of RuBP carboxylase reported in the early (reversible) part of senescence. In later (irreversible) part of senescence, 80% decline in the specific activity of RuBP carboxylase was noted.	20
Hordeum vulgare	The activity of this enzyme was more sensitive than the primary photochemical reactions.	78
Glycine max	Leaf senescence-associated decline in RuBP carboxylase activity was not altered by desinking treatments.	79
Triticum aestivum	No change in the specific activity of this enzyme, on either milligram protein or RuBP carboxylase basis, was observed until late senescence.	80
Triticum aestivum	Decline in RuBP carboxylase activity was recommended as a parameter to screen genotypes for altered senescence patterns.	81
Hordeum vulgare	Loss of RuBP carboxylase probably represented the primary event responsible for the decline in photosynthesis during leaf senescence.	82
Glycine max	RuBP carboxylase activity was not closely correlated with the changes in photosynthesis.	83
Triticum aestivum	RuBP carboxylase activity declined in a differential manner in leaves and reproductive parts (i.e. awns, glumes, grains) of two field-grown cultivars.	84
Cicer arietinum	Leaf RuBP carboxylase activity shown to decline during pod development in a number of field-grown cultivars.	85
Glycine max	RuBP carboxylase and chlorophyll content declined concomitantly with photosynthesis during senescence.	86
Oryza sativa	Indicated that the level of RuBP carboxylase protein can be a limiting factor in photosynthesis throughout the life span of the leaf.	87
Oryza sativa	Documented that both the amount of RuBP carboxylase protein and leaf conductance reflect the change in photosynthesis during senescence.	88
Triticum aestivum	RuBP carboxylase activity declined continually with ageing, but *in vivo*-activated activity has a minimum of three-fold greater than needed to account for photosynthetic activity.	89
Triticum aestivum	Decrease in chloroplast number and the loss of RuBP carboxylase activity were not correlated.	90
Oryza sativa	This protein degraded rapidly during the early stage of senescence in the nitrogen-sufficient leaf, whereas its degradation proceeded almost constantly in the nitrogen-deficient leaf.	91
Cajanus cajan	Loss of RuBP carboxylase activity from senescing leaves was correlated to the decline in leaf-nitrogen and built up of seed nitrogen. Leaf RuBP carboxylase activity was affected by desinking treatments.	92
Cajanus cajan	Certain modifications in the methodology for measuring *in vitro* RuBP carboxylase activity were suggested for documenting ontogenetic stage-dependent changes.	93
Cicer arietinum	Decline in leaf RuBP carboxylase activity was temporally correlated to pod-nitrogen status. The desinking treatments affected the course of senescence associated loss of leaf RuBP carboxylase activity.	94
Oryza sativa	Indicated that the rate of CO_2 assimilation under ambient air conditions was limited during entire leaf span by the RuBP carboxylase activity. This limitation was suggested to be due to the involvement of enzyme activation.	95
Triticum aestivum	Leaf senescence at elevated temparature (35° C) resulted in greater inactivation of RuBP carboxylase than control (25° C). It was also suggested that the loss in Chl and RuBP carboxylase activity is triggered simultaneously in senescing leaves.	43
Triticum aestivum	Showed that photosynthesis did not increase in proportion to the amount of RuBP carboxylase achieved by nitrogen application.	96

Table 1. (*Continued*)

Triticum aestivum	Neither RuBP carboxylase activity nor the rate of electron flow (Hill reaction) can be said to be the cause of the decline in the rate of assimilation during senescence.	97
RuBP carboxylase degradation		
Triticum aestivum	This protein appeared to be preferentially degraded. Proteinase activity, checked against purified RuBP carboxylase protein, increased sharply during the period of most rapid loss of RuBP carboxylase.	24
Triticum aestivum	The specific activity and number of active sites per molecule of RuBP carboxylase did not change during senescence. The rise in proteolytic activity coincided with the onset of preferential loss of immunologically recognizable RuBP carboxylase.	25
Hordeum vulgare	Showed hydrolysis of ^{14}C-labelled RuBP carboxylase by three endoproteinases (two partially purified).	23
Triticum aestivum	Proteolytic activity associated with the degradation of RuBP carboxylase localized in vacuoles. Photosynthesis declined prior to RuBP carboxylase which may indicate that RuBP carboxylase is not the primary limiting factor during leaf senescence.	98
Avena sativa	Demonstrated enhanced proteolysis of RuBP carboxylase by measuring *in situ* and *in vitro* proteolytic activity in crude extracts of senescing leaf segments.	99
RuBP carboxylase synthesis		
Triticum aestivum	The rate of synthesis of RuBP carboxylase falls sharply relative to the average rate for all proteins, following full leaf expansion. There was a coordinated decline in the synthesis of subunits of RuBP carboxylase during ageing.	100
Triticum aestivum	An extension of the previous work[100] in which it was shown that the decreasing abundance of the translatable mRNAs of both subunits of RuBP carboxylase were coordinated.	101
Sinapis alba	Reported difference in the time course for the loss of RuBP carboxylase and SSU-mRNA in senescing cotyledons. Decline in RuBP carboxylase activity appeared to be phytochrome-dependent.	71
Phaseolus vulgaris	Decline in synthesis of LSU and SSU polypeptides shown. Indicated that the transcript level for rbcL remain a constant proportion to total RNA throughout senescence. By contrast, transcript levels for rbcS comprise a progressively decreasing proportion of total RNA.	28

protein, another crucial point is to show that intact chloroplasts possess proteolytic activity. Appreciable peptide hydrolase activity associated with chloroplasts has been noticed in barley mesophyll protoplasts[26]. Isolated chloroplasts from pigeonpea leaves when incubated at high temperatures, were shown to liberate amino-nitrogen linearly for two hours, thereby implicating that the chloroplast-borne proteases could have a significant role in the turnover of chloroplast proteins[4].

It is important for a senescing leaf cell to not only enhance proteolysis but also effectively curtail the rate of protein biosynthesis so that amino-nitrogen is not reutilized. Numerous studies document that the senescing leaves have a lower rate of protein biosynthesis (see Table 1). Recent evidence suggests that the level of mRNAs for both large subunit (LSU) and small subunit (SSU) of RuBP carboxylase is also concomitantly reduced[27]. When equal amounts of RNA from leaves of naturally senescing second leaf of wheat (10, 13, 16, 19, 22 and 24 days after sowing; full expansion was on day 13) were probed with ^{32}P-labelled DNA specific for LSU or SSU of RuBP carboxylase, both LSU and SSU mRNA showed an actual decline on Northern blot. From these observations, it can be inferred that the changing pattern of synthesis of LSU and SSU of RuBP carboxylase is controlled directly in terms of the mRNA population and not by some form of translational control. Recently, senescence-associated decline of LSU and SSU of RuBP carboxylase has also been followed in *Phaseolus vulgaris*[28]. In this work, the decline in LSU and SSU was shown by Western blotting and, subsequently, gene-specific probes were employed to mark the concomitant changes at the gene level. Dot- and Northern-blotting data indicated that down-regulation of nuclear encoded SSU correlated with an apparent selective decrease in the abundance of corresponding transcripts. By contrast, the decline in the abundance of LSU appears to correlate with a general decline in total RNA levels.

The decline in steady-state level of a given mRNA can potentially be achieved by the following two approaches: first, by its increased rate of RNase-mediated degradation and secondly, by reduction in their rate of biosynthesis through reduction in transcriptional activity of the corresponding gene(s). If the first possibility is true, it is prerequisite that the progress of senescence should lead to formation of such 'reactants' which could possibly affect the half lives of these mRNAs. Several *in vitro* methods for the

assessments of the half lives of RNA molecules in response to various extrinsic and intrinsic factors have been standardized for animal systems[29]. But, so far, such methods have not been developed for the study of the plant senescence.

The transcriptional mechanism of RuBP carboxylase synthesis has been followed vigorously in recent years. It is not in the scope of this article to give all concerned details and, therefore, only the most relevant points are mentioned. It has been revealed that the SSU of RuBP carboxylase is synthesized by a family of genes[30,31]. Furthermore, it has been shown that the sequences present upstream to these genes govern the rate at which the mRNA for the SSU of RuBP carboxylase are synthesized, in specific tissues and in response to specific light conditions[32]. These upstream sequences called the *cis*-acting elements, in turn, interact with certain protein factors, called *trans*-acting factors, and this binding is thought to be the master control responsible for tissue as well as light-specific expression of this polypeptide[33]. The questions thus arise: How is senescence-related reduction in the rate of transcription of SSU genes achieved? Are *trans*-acting factors formed at the same rate in healthy and mature leaves? Could senescence affect the affinity of these *trans*-acting factors towards their binding to the *cis*-acting sequences? However, as yet, these questions have only been addressed to a limited extent.

In addition to RuBP carboxylase, several other enzymes involved in dark fixation of carbon dioxide together contribute significantly to the nitrogen balance of the leaves[34]. Senescence has been shown to adversely affect the activities of these enzymes too[35]. However, the detailed mechanism underlying their losses has not been worked out to any appreciable depth. Therefore, it is not possible to comment whether decline in activities of these enzymes is crucial to the overall rate of photosynthesis in senescing leaves.

Photosynthetic light reaction and leaf senescence

The components of the light reaction of photosynthesis, such as various thylakoid proteins, electron transport carriers, several enzymes and subunits of ATPase contribute significantly towards chloroplast and leaf nitrogen status[34,36]. Besides nitrogen, several other nutrients (Mg^{++}, for example) can also be recovered from the remobilization of these components[37]. Therefore, senescence-associated remobilization of nutrients is expected to involve the hydrolysis of light reaction-related proteins too, though, their hydrolysis may not be as predominant and preferential as that of RuBP carboxylase. We shall now assess effect of senescence on the light reaction of photosynthesis. However, before doing that, it is important to appreciate that the photosynthetic light reaction is a complex of several serial reactions. It is important to dissect this complexity in order to reveal the crucial steps. Fortunately, it is possible to undertake analysis of various partial reactions of photosynthesis because several artificial electron acceptors, donors and inhibitors have been characterized[38] which enable to study the sensitivity of various components individually.

The photochemical electron transport activities are reported to be adversely affected during leaf senescence. For instance, the rate of coupled, non-cyclic electron transport from water to methyl viologen ($H_2O \longrightarrow MV$), representing whole chain electron transport activity, has been shown to decline in several plant systems[39-43]. Theoretically, factors such as change in characteristics of light harvesting complexes, loss of the photosystems and carriers of electron transport chain can potentially result in reduction of whole chain electron transport activity. It has been reported that the antenna size associated with each reaction centre in *Phaseolus vulgaris* leaves remains stable during senescence[44]. Decline in structural apoprotein content of the light harvesting protein complex (LHCP) has been marked by Western blot analysis[45]. A more rapid loss of reaction centre complex of photosystem II (PSII) than light harvesting complex II has also been noted[46]. In addition to whole chain, the reduction of both photosystem I (PSI) [reduced 2, 6-dichlorophenol indophenol ($DCPIPH_2$) $\longrightarrow$ MV or TMPD $\longrightarrow$ MV)] and PSII ($H_2O \longrightarrow$ ferricyanide (FeCN), in presence of oxidized p-phenyl-enediamine (PDox) electron transport activities has been convincingly shown in senescing leaves[47]. In a few cases, the Western blot analysis has revealed that the representative subunits of these photosystems also decline *per se*. However, the behaviour of all polypeptides associated with these photosystems may not be the same because no change in amount of PSI reaction centre polypeptide was noted by Western blot analysis of senescing oat and bean leaves[48] and the synthesis of 32 kD herbicide-binding or Q_B protein has been reported to continue during senescence[45].

On a comparative basis, it has been found that the rate of decline of the whole chain electron transport activity is much higher compared to the rate at which the two individual photosystems decline[41,47]. Therefore, it has been suggested that the impairment of electron flow between the two photosystems, more importantly, limits non-cyclic electron transport in older leaves. A great deal of effort has been made to study various electron carriers which functionally join the two photosystems. According to Holloway *et al.*[49], the rate of electron flow from water to MV or FeCN is limited by plastoquinone (PQ) prior to leaf maturity, whereas during senescence, rate-limiting step was on the

reducing side of the PQ, probably at electron transfer from PQ to cytochrome (Cyt) b_6/f. Senescence-related relatively faster decline in level of different components of Cyt b_6/f complex has also been marked by Western blot analysis[45,48]. The amount of plastocyanin (PC) has also been found to be reduced in senescing leaves as shown employing KCN, an antagonist of the PC[42]. No change in the amounts of polypeptides of chloroplast coupling factor was noted in senescing oat and bean leaves by Ben-David *et al.*[48], whereas formation of α and β subunits of ATPase has been found to decline in senescing bean leaves by Roberts *et al.*[45].

As a result of the above-mentioned alterations in the electron carriers, and particularly with the viewpoint of the fact that the inter chain electron transport components are affected to a greater extent than the two photosystems, a situation leading to inefficient electron transfer from the reduction side of PSII towards the reaction centre of PSI can possibly arise. This was indeed shown to be the case by experiments involving studies on pH optimum in basal as well as methylamine (an uncoupler) induced electron transport activities[42,50-52].

Though extensive losses in photosystems and electron transport components are documented in the literature, little has been understood regarding the mechanistic details for the same. The proteinases which can potentially act on the electron transport components have not yet been identified and characterized and state of literature in this respect is in contrast to that of RuBP carboxylase. The case of RuBP carboxylase is unique because getting this protein in large amounts for experimental purposes is not a problem. However, to purify the electron transport components at the preparative scale is a difficult task. Likewise, the information available on biosynthesis of the electron transport components is scanty. It is however hoped that the characterization of the rate of synthesis of the electron transport components should now be possible because genes for several of these proteins have been cloned, and as a matter of fact, the entire sequence of the chloroplast genome of three plant species (*Marchantia polymorpha, Oryza sativa* and *Nicotiana tabacum*) is known[53-55]. The levels of mRNAs of various electron transport carriers can be followed from young and senescent leaves using their respective complementary sequences as probes. An attempt in this direction has been made recently by Bate *et al.*[28] in senescing *Phaseolus vulgaris* leaves, employing gene-specific probes. This study undertook analysis of chlorophyll *a*, *b* binding protein (cab), a nuclear gene encoding the 26 kD protein of LHCP and psbA, a chloroplast gene encoding the D1 protein of PSII. As previously mentioned for LSU and SSU[28], the data for these probes indicated that decline in synthesis of nuclear encoded LHCP correlate with an apparent selective decrease in corresponding transcript. On the other hand, decline in synthesis of D1 protein of PSII appears to correlate with a general decline in total RNA.

We have, so far, described senescence-related alterations in the components of electron transport activity. The overall efficiency of the light reaction would also be affected with a decline in the amount of the photosynthetic pigments, particularly chlorophylls. The loss in amount of chlorophyll is considered to be the most pronounced indicator of senescence[56], and barring a few exceptions[57], can be employed as a reliable parameter to study senescence. The details on this aspect have been reviewed earlier by several workers and are therefore not described here.

Gene expression changes in senescing leaves

The above-mentioned studies have mainly dealt with the specific changes in the light and dark reaction components of photosynthesis. However, these changes reflect the reactions which are *fait accompli*. What causes initiation of senescence? Which gene products accumulate during the early phase of senescence? The answers to these questions are important, particularly in view of a possible genetic manipulation of the senescence programming. With the current upsurge in molecular techniques of gene isolation, gene cloning, RNA isolation, *in vitro* and *in vivo* protein labelling and their separation by gel electrophoresis, it is becoming increasingly possible to address oneself to the molecular aspects of chloroplast and leaf senescence.

Earlier studies[58] on identification of specific polypeptides associated with chloroplast senescence were made employing one-dimensional sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Subsequently, the senescence-associated appearance of new mRNA species has been analysed in terms of their protein products employing both one-dimensional SDS-PAGE and two-dimensional isoelectric focusing + sodium sulphate dodecyl-polyacrylamide gel electrophoresis (IEF + SDS-PAGE)[59-62]. These attempts have successfully shown several polypeptides which specifically appear during senescence. The accumulation of mRNAs coding for certain specific molecular weight polypeptides in intact chloroplasts has been shown in senescing barley leaves[63]. Furthermore, it has been reported that these senescence-associated polypeptides are distributed among various subfractions of chloroplast such as stroma, envelope and thylakoids, in a specific manner[64].

In general chloroplast gene expression is thought to be controlled at the level of transcription[65,66] as well as translation[67,68]. It has also been established that the expression of most of the nuclear photosynthetic genes

is transcriptionally regulated[69]. Plastid photosynthetic genes are generally thought to be regulated post-transcriptionally during development, either through differential transcript stability or at the level of translation[70]. In a few instances, evidence for controlled gene expression in senescing chloroplasts has been obtained[58,71,72]. However, detailed mechanism responsible for this controlled gene expression is not yet known. Recently, data of Bate *et al.*[28] have provided evidence that the generalized scheme of nuclear and chloroplast gene regulation mentioned above may be true for senescing leaves also. Furthermore, the cooperation between the nuclear and plastid genome has been intensively studied in recent years and it is generally accepted that the plastid development is controlled by the genetic information encoded in the nucleus to a large extent[68,73]. On the other hand, it has also been shown that plastids are involved in controlling extraplastidic reactions[74]. However, the importance of nuclear–plastid interactions during senescence has not received much attention thus far.

Conclusions

This article has highlighted the current understanding regarding how the rate of photosynthesis as well as the overall photosynthetic efficiency is limited in senescing leaves. Towards this end, several attributes concerning cellular, biochemical, bioenergetic and molecular expressions of the metabolism of senescing leaves have been presented. Much emphasis in this context has been placed on metabolism of RuBP carboxylase protein and electron transport carriers, particularly plastocyanin and cytochromes. However, the article has failed to achieve its desired objective of pinpointing the 'reaction(s)' which possibly rate-limit photosynthetic process in senescing leaves. This failure is due to rather diffuse understanding of this subject even till date, despite all efforts. A more focused understanding of these changes is essential in order to devise strategies for delaying the initiation of the senescence programme. The functional role of polypeptides which appear rather rapidly in senescing leaves is still to be elucidated. Information on identification of genes which govern novel polypeptides marked at senescence stage is in infancy. With the viewpoint of emerging gene engineering technologies, some of these aspects are expected to gain momentum in the near future.

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Systemic autoimmune diseases: Possible involvement of superantigens in the abnormal immune response

M. S. Aryan Namboodiri

Department of Biochemistry, Indian Institute of Science, Bangalore 560 012, India

Multiple autoantibody responses associated with systemic autoimmune diseases like systemic lupus erythematosus and rheumatoid arthritis were once considered to be due to the nonspecific activation of the immune system; possibly by polyclonal B-cell mitogens. But unlike a mitogen-induced response, autoimmune response associated with these diseases was found to be class II major histocompatibility complex (MHC) restricted and T-helper cell regulated. So it is proposed that rather than acting alone, the mitogens may be synergistically acting along with a weak self-antigen to give a specific response. However, recently a group of antigens have been identified, which eliminate the requirement of such a synergistic action. They give antigen-specific and polyclonal antibody responses, similar to the autoimmune response in the above diseases, by employing T-helper cells and class II MHC. These proteins termed 'superantigens' bind to T-helper cells bearing a limited set of β-chain variants of T-cell receptor. Though superantigens require class II MHC molecules for presentation to T cells, the binding is to the non-polymorphic region of MHC unlike the conventional antigens which bind to the polymorphic region. These antigens explain the initiation of autoimmune response by the non-specific activation of T-helper cells.

Autoimmune diseases comprise a group of disorders where there is nothing apparently in common other than an exaggerated immune response to one or more of the self-antigens[1]. Historically autoantibodies were considered to be something abominable and the theory of sequestered antigens and forbidden clones was proposed[2,3]. Recent studies however show that autoantibodies and autoreactive T cells are normal[4-7]. Now the question arises as to what contributes to the diseases.

Autoimmune diseases are classified into organ-specific and systemic diseases[8]. In organ-specific diseases the autoimmune response is directed against some unique antigens present on one or more of the tissues, whereas in systemic autoimmune diseases like systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) the autoimmune response is found to be multiple autoantigens. Since the antibody response in these diseases is not very highly specific, it was considered that the immune response is due to the non-specific activation of the immune system.

Nonspecific immunostimulation

Though antigen-dependent stimulation is the convention, there exist alternate mechanisms for the activation of the immune system. Substances which activate T and B cells in this way are called mitogens[9]. Many carbohydrate-binding proteins (lectins) possess the potency to activate T and B cells. B cells can also be activated by anti-immunoglobulin and B-cell mitogens (lipopolysaccharides, dextran sulphate, etc). Induction of antibodies by these compounds is not specific and results in multiple antibodies including autoantibodies[10,11]. It is speculated that polyclonal B-cell mitogens (PBMs) may be responsible for systemic autoimmune diseases[12]. Administration of multiple immunostimulators in mice brings about severe but transient autoimmune syndrome[13,14]. Furthermore, circulating PBMs have been detected in RA and SLE patients[15,16]. But there are many drawbacks in extending this PBM-induced model to the actual disease. Autoantibodies induced by potent B-cell stimulators like epstein barr virus (EBV) are predominantly IgM (ref. 17) whereas, in these diseases the pathogenic antibodies are of other isotypes. Potent